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X

THE CEPHALOSPORIUM DISEASE OF ELMS

BY

DON BAKER CREAGER

WITH SIXTEEN PLATES

PUBLISHED BY
THE ARNOLD ARBORETUM OF HARVARD UNIVERSITY
JAMAICA PLAIN, MASS., U.S.A.
1937

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TABLE OF CONTENTS

INTRODUCTION 5

REVIEW OF LITERATURE 5

SYMPTOMATOLOGY 9

A. External symptoms 10

B. Internal symptoms 14

C. Signs 21

ETIOLOGY 21

A. Culture studies 21

B. Morphological studies 33

C. Inoculation studies 44

D. Life history studies 64

CONTROL 73

A. Pruning tests 73

B. Fungicidal and insecticidal tests 76

C. Control recommendations 80

SUMMARY AND CONCLUSIONS 81

ACKNOWLEDGMENTS 83

LITERATURE CITED 84

EXPLANATION OF PLATES 86

THE CEPHALOSPORIUM DISEASE OF ELMS

INTRODUCTION

A threatening disease of elms, now commonly known as the "Dutch elm disease", was discovered in Europe in 1919 and in the United States in 1930. The discovery of this disease in the United States at once impelled the attention of American phytopathologists to the diseases of elms in general. Their observations soon revealed the existence of two native diseases very similar to that of the Dutch elm disease. One of these, to which very soon the name "Cephalosporium disease" was applied, has been found to exist practically throughout the natural range of the American elm and to be the cause of extensive, hitherto baffling losses in nurseries, plantations and natural stands. Some phases of it have been studied previously by several other investigators. But these studies have been so restricted or incidental that scientific knowledge of the disease has remained very incomplete. Because of its own relative importance and because of the help that might be afforded in the effort to save America's elms from the Dutch elm disease a monographic study of the Cephalosporium disease of elms was undertaken by the writer. The results of more than three years of study both in the field and in the laboratory are presented in this paper.

REVIEW OF LITERATURE

Verrall (1930) published a brief account of an elm disease observed in Minnesota in 1929. The disease was causing considerable die-back of twigs and branches in both nursery stock and older trees. The causal agent appeared to enter through the tips of branches and work down through the wood and bark causing the wood to become brown-streaked. The wilting of leaves and the formation of cankers were also referred to. No fructifications on the diseased elms were found, but in culture, groups of abortive pycnidia containing minute, hyaline, elongated spores developed in stromata. He placed the organism in the Sphaeropsidales, possibly near *Cytosporella*. Preliminary inoculation tests indicated that the fungus isolated was the cause of the disease. However, it was pointed out that, since weakened trees were used,

these tests did not show satisfactorily how virulent the pathogene was and to what extent it would develop in healthy trees.

The director (1931) of the Connecticut Agricultural Experiment Station, in his annual report for the year ending October 31, 1930, stated that an elm disease found in New Haven was being investigated. A fungus was isolated which caused slight injury in a few cases when inoculated into young elms. Its identity had not been determined.

May (1931) reported a new disease of elm caused by a fungus which was tentatively classified as a species of *Cephalosporium.* The fungus was isolated from specimens of elm suspected of having the Dutch elm disease; these were collected in Ohio, Iowa, Missouri, New York, and Washington, D. C. Seven out of fourteen young elms inoculated with this fungus showed symptoms of wilting, yellowing and browning of leaves, blackening of the bark above point of inoculation, and browning of cambium area and current growth of sapwood. On potato dextrose agar the aërial mycelium of the fungus was first white and later light brown; on this medium small heads of hyaline, ellipsoid spores were formed on ends of short conidiophores.

In a later report by the director (1932) of the Connecticut Agricultural Experiment Station it was stated that the fungus isolated from diseased elms in New Haven five years previously was the same as that found in Ohio by Curtis May, although inoculation tests revealed it to be a weaker parasite than the Ohio strain.

Harris (1932) mentions isolating cultures of *Cephalosporium* from elms in Illinois, but stated that they were not all identical with the *Cephalosporium* described by May.

Liming (1933) reported that in three seasons 1,407 elms suspected of having the Dutch elm disease were cultured at the Dutch Elm Disease Laboratory at Wooster, Ohio; and of these 336 yielded a fungus referred to *Cephalosporium.* He stated further that trees affected with *Cephalosporium* show an increase in the diseased condition the second year.

Beattie and Gravatt (1933) recorded that the wilt disease of elms caused by *Cephalosporium* sp. is found widely distributed over the Lake States and Eastern States, and a few cases in Middle Western States.

Goss and Frink (1934) published a much more extensive account of the Cephalosporium disease than any which preceded

it. Goss first observed this disease in Nebraska in 1931. By a comparison of cultures the pathogene was found to be the same as that referred to *Cephalosporium* sp. by May. The symptoms of the disease were recognized as somewhat similar to those described by Verrall (1930) for the disease of elm reported in Minnesota. No pycnidia were produced by the Nebraska fungus. Symptoms of artificially inoculated plants similar to those observed by May (1931), were described and in part illustrated. In a study of the pathological anatomy tyloses or tylose-like bodies, as well as yellowish gum-like substances and hyphae, were recognized in the vessels of infected stems and leaf petioles. The characteristics of the hyphae, spore heads and spores as they appeared in culture were described and figured. Goss and Frink also studied the effects of temperature and hydrogen-ion concentration upon the growth of the fungus in culture. The optimum temperature for growth was found to be 25 degrees Centigrade. It was pointed out, however, that the data obtained by the method of growing giant colonies in petri-dishes and measuring radial growth, as employed in these studies, did not indicate the effect of temperature upon the total growth of the organism; the thickness of the colony varied with the different temperatures. The optimum pH for growth was found to be in the neighborhood of pH 6 to pH 7. There was a marked tendency for the growth of the fungus to cause the reaction of the medium to shift towards neutrality from both higher and lower conditions of pH. In inoculation tests, using young white elms, infection was obtained by inserting the inoculum into roots and trunks through wounds, by hypodermic injection in trunks, by dipping roots into spore suspensions and by atomizing tops with spore suspensions. The authors state that infection resulted in several plants whose tops were atomized "regardless of whether or not the twigs had been injured by pin pricks before atomizing". Infection in the supposedly uninjured plants was thought to have occurred "probably through accidental injuries rather than through the leaves". Limited tests, using Chinese elms, yielded negative results. The rôle of insects in dissemination and inoculation was suggested, reference being made to those which injure stems. Neither the external sources of inoculum nor the natural infection courts had been determined; however, it was pointed out that infection had occurred in new branches each year by penetration from the outside as observed in a diseased tree which was studied in some

detail. Apparently no control tests were conducted, but the removal of badly infected trees and pruning out of affected portions of trees slightly infected were recommended as possible control measures.

The Cephalosporium disease was reported from New Jersey in 1933 (Plant Disease Reporter, 1935); and soon after the publication of the bulletin by Goss and Frink (1934) the New Jersey Agricultural Experiment Station (Nursery Disease Notes, 1934) brought the disease to the attention of Eastern nurserymen.

The disease was reported from Massachusetts by Boyd in 1934 and in the following year Davis stated that the Cephalosporium disease was becoming more prevalent in Massachusetts and other parts of New England and was of most serious concern.

Tehon (1934) considered the Cephalosporium disease to be first in importance among the wilts of elms in Illinois; yet, contrary to conclusions by Verrall (1930) in Minnesota and Goss and Frink (1934) in Nebraska, he was of the opinion that it was of practically no importance in nurseries. The only exception was that of a few blocks of Chinese elms which he had found to be completely infected.

A second fruiting stage, pycnidia, was reported for the fungus causing Cephalosporium disease of elms by May (1934) at the annual meeting of the National Shade Tree Conference in 1934.

Regarding the prevalence and distribution of the disease in America, Verrall (1935) stated that, out of 16,921 specimens of diseased elms sent to the Dutch Elm Disease Laboratory during the summer of 1934, 28.2 per cent (or 4,769) yielded *Cephalosporium* in culture. At that time the Cephalosporium disease was found to be prevalent in native stands and planted elms in 20 States and in Canada, namely, from Oklahoma northward to North Dakota and eastward to Virginia and Nova Scotia. The disease was said to be causing much disfiguration and occasional death of trees.

Based on the characteristics of the pycnidial stage, Verrall and May (1937) have recently named the pathogene of this disease *Dothiorella ulmi*, n. sp. It was proved by cultural methods that this fungus produces both spore heads (Cephalosporium stage) and pycnidia. Pycnidia were found to be sparsely developed on newly formed cankers on twigs and small branches; they have been found on specimens of elm collected in Virginia, Ohio, New Jersey, and Oklahoma. Concerning the prevalence and distribu-

tion of the disease these writers report that 57,347 specimens from elms suspected of having the Dutch elm disease were submitted to the Dutch Elm Disease Laboratory from 1930 to 1935, and that 42 per cent (or 24,086) of these yielded *Dothiorella ulmi* in culture. These specimens were collected in 28 States, the District of Columbia and Canada. They state that the disease is common wherever elms occur, from Virginia to Oklahoma and Canada, and that a few infected specimens were received from Colorado and Montana. Most of the specimens which were found to be affected with the Cephalosporium disease were supposedly American elms but a few were slippery elms (*Ulmus fulva* Michx.). By comparison of cultures these writers came to the conclusion that the three diseases independently reported from Minnesota (Verrall, 1930), Connecticut (Anonymous, 1931), and Ohio (May, 1931) were all the Cephalosporium disease.

SYMPTOMATOLOGY

The symptomatology of the Cephalosporium disease of elms has received some attention at the hands of Verrall (1930), May (1931), and Goss and Frink (1934); but their descriptions have been brief, more or less general, and deficient with respect to many important features. It is especially noteworthy that no one has treated the subject from the standpoint of the disease as it occurs in the field. I am, therefore, presenting in rather complete detail an account of the symptoms and signs of the Cephalosporium disease of elms as I have seen them in the course of three years intensive field and laboratory study. This is prefaced by an account of the general appearance of a severely diseased tree.

An infected tree always exhibits dead twigs and branches which have been killed back from their tips (Plate I). Additional branches become infected and more are killed each season. The sudden blighting of leaves and young twigs, often over whole branches, is a common occurrence. Blighting is a striking symptom and is one which readily attracts the attention of the most casual observer (Plate II). Also, many leaves may become yellowish and fall from the tree prematurely. Abundant suckering or shoot formation usually occurs along the badly infected larger branches and trunk. The cambium area and underlying sapwood of infected stems exhibit brown-discolored streaks or more diffuse, broader bands. Small trees may be killed rather quickly; larger

ones may be very much deformed before they are eventually killed (Plate II). The details of the various external and internal symptoms and signs of the Cephalosporium disease will now be considered.

A. EXTERNAL SYMPTOMS

Leaf lesions

All leaf symptoms referred to by previous investigators were either direct expressions of infection resulting from the ingress of the pathogene from the infected stem through the petiole or were merely indirect manifestations of stem infection. My investigations have revealed the fact that ingress of the fungus takes place through wounds in the leaves and it is here that the earliest symptoms of the disease are expressed (Plate III). These symptoms were briefly referred to by the writer (1935) in a previous preliminary report. In the vicinity of central and southern New England the first symptoms of incipient infection in the leaves begin to appear about the middle of June as localized yellowish areas near wounds and darkened veins extending out from the yellowish lesion (Plate III, figure 1). The yellowed area increases and the portion near the wound dies and turns brown (Plate III, figure 2). As the lesion enlarges it may appear irregularly zonate, characterized by several successive areas of different shades of brown surrounded by the yellow margin. Under conditions favorable for an abundant supply of inoculum, for spore dissemination and for infection, hundreds of such lesions may appear on an individual tree. Indeed, there may be several infected leaves on a single twig tip, and several lesions may appear on a single leaf. In three weeks or more after the initial symptoms appear, infected leaves drop, the lesions at that time rarely involving more than one-half of the leaf blade.

Die-back

The symptom complex resulting from the successive defoliation and dying centripetally of the twigs and branches from their tips is commonly referred to as "die-back". The symptom of die-back makes its initial appearance soon after the primarily infected leaves drop. Beginning with those leaves nearest the infected scar, the leaves drop one by one until finally the tip is completely defoliated (Plate III, figure 3). If the infected scar happens to be located some distance away from the tip of the twig, the next leaf above on the same side of the stem is usually the next to fall, and

for a short time defoliation may be restricted to that side of the twig. The progress of defoliation in such a case is also more rapid above the infected leaf scar than below. Before such a leaf drops, the petiole may curve slightly downwards and a yellowish discoloration of the blade may develop from the base of the leaf outwards along one or both sides of the midrib; however, the leaf usually drops before the yellowing reaches the leaf tip or its lateral edges. Wilting of such leaves has rarely been observed.

Running parallel or following closely behind the gradual defoliation of the tip of the twig, the axillary bud immediately above the scar from which a primarily infected leaf had fallen becomes shriveled, dries and eventually falls off. At about the same time a blackening of the bark begins at or near the infected scar. The blackened area may be a slightly sunken continuous lesion from the very start or may at first appear as small flecks over a limited area upwards and downwards from the scar. In either case the blackened area increases and may finally involve the entire twig tip, ending gradually in the normal greenish or olive-brown bark below. If the twig is infected some distance from the tip the upper portion may be killed before the blackened area reaches it. In such an instance the black area may extend diffusely above into the reddish brown bark of the uninfected killed tip.

Defoliation and dying back of infected twigs proceed slowly downwards. In most cases a limited portion only of the current season's growth is involved before the approach of the winter dormant season. Some cases have been observed where infection had not progressed beyond the immediate vicinity of the leaf scar or was restricted to a limited streak along one side of the twig at the time of normal leaf fall. Thus a twig becoming infected through leaf lesions may not be killed until the following season.

When the infected twig comes out of dormancy the following spring additional symptoms of die-back soon appear. In the vicinity of central and southern New England these renewed symptoms begin to appear about the middle of May. Some or all buds near the tips may fail to open, others may be killed soon after opening, while still others farther down may produce side branches, these only to be killed in succession as the area of infection increases (Plate III, figure 4). Such branches continue to die back indefinitely and the picture becomes more complicated as additional new twig tips become infected each season.

BLIGHT

Blight of young shoots and leaves occurs commonly throughout the earlier part of the growing season. The first blight to appear involves the young shoots which become infected as a result of continued dying back of previously infected twigs and branches in early spring. The leaves on the very young shoots suddenly wilt, roll upwards as they rapidly dry, turn brown and cling to the branch indefinitely (Plate III, figure 4). On somewhat older shoots the leaves may only slightly droop, become yellowish or mottled yellowish-green, and begin drying and browning at the margins before they are entirely killed. Wilting and death of the leaves may be accompanied by a drooping of the affected shoot and in the process of drying tender tips commonly curve into the shape of "shepherd's crooks".

Larger branches, which become infected through diseased lateral shoots or through diseased main branches from which they arise, often suddenly exhibit marked symptoms of blight. As on smaller shoots, the leaves suddenly wilt, quickly dry, curl, turn brown and cling to the twigs. These large branches of blighted brown leaves stand out in marked contrast to the healthy green foliage of the same or near-by trees. Sometimes whole tops of smaller trees are blighted (Plate II, figure 2). Protracted rainy periods seem to be especially favorable for the development of blight on these larger branches during the last part of May and June, because the symptoms often appear soon after.

Later in the season, after the leaves have become hardened, blight does not appear. Instead, the leaves on infected branches droop slightly or not at all, become yellowish to brown-discolored from their bases towards their tips and soon drop. The yellowish discoloration is most intense at the base of the leaf and along one or both sides of the midrib, gradually fading out towards the tip and the margin of the blade; very often the discoloration involves only one side of the leaf, especially in its early stages of yellowing. Some leaves may absciss at this early stage when they are only slightly discolored, while others may remain somewhat longer when more advanced symptoms of death and browning of the blade occur. Death and browning of the blade sometimes follow the course of the preceding yellow discoloration, but often the reverse occurs, the affected blade dying and browning from its tip and margin towards the base and midrib.

Defoliation

The ground beneath infected trees may be continually littered with fallen leaves, beginning late in June and becoming especially noticeable during July and August. Of course, premature defoliation is not specific for the Cephalosporium disease, as there are several other diseases of the leaves and stems, as well as injuries and unfavorable environmental conditions, which cause the same phenomenon. In case of the Cephalosporium disease, premature defoliation approaches a specific symptom only when the more detailed symptoms of the fallen leaves are taken into account. In general, one finds three types of fallen leaves; first, brown curled leaves which were blighted earlier in the season; second, green or slightly yellowish leaves bearing one or more primary leaf lesions originating at wounds; and third, leaves with a yellowish discoloration extending outwards from the base along one or both sides of the midrib towards the tip, or with more advanced symptoms of death and browning. This third type comes from infected twigs after the leaves are too hard to become blighted. Leaves of both the second and third types, with their only partially killed blades and striking yellowish and brown necrotic lesions are quite conspicuous when littered over a well kept lawn.

Twig abscission

The ground beneath an elm affected with the Cephalosporium disease not only may be littered with fallen leaves, but with small twigs and branches as well. Many small branches and twigs, up to a quarter of an inch in diameter, along the sides of larger infected branches, often abnormally absciss. Quite commonly infection proceeds along one side of a branch, affecting twigs on that side and not the other. Such affected side branches frequently absciss before they are entirely killed.

Cankers

The appearance of flattened or sunken elongated cankers on the sides of infected branches is quite common in elms affected with this disease. Preceding the formation of definite cankers, a flattened discolored streak may appear along one side of the branch, and in many instances abscission of twigs may also occur along the infected side (Plate IV, figure 1). In young branches the bark over the infected area sometimes becomes blackened, occasionally beginning as flecks centered about lenticels; but in older branches the greenish shade of the characteristic olivaceous

brown of the healthy bark may disappear and turn to a somewhat lighter or darker shade of brown. Eventually the lesion may become sunken and the bark at the edges will crack.

Cankers more commonly occur on branches a quarter of an inch to two inches in diameter and rarely on branches exceeding three inches. They can often be traced to killed or badly infected lateral branches or forks. Large cankers sometimes extend down along larger branches, or even the trunks of small trees, to a distance of three feet or more. In the more advanced stages it seems quite probable that various saprophytic and wood-rotting fungi, which are quite frequently found fruiting on the affected bark, may aid the primary pathogene in canker formation.

Suckering

Infected trees commonly exhibit an abnormal abundance of slender whip-like suckers. The dying back of the branches from their tips stimulates sucker formation below the infected region. Since infection proceeds rapidly towards the tip and rather slowly downwards, these suckers have an opportunity to make considerable growth before they, too, are infected. In many instances suckers are killed the same season in which they grow out; some, however, are not killed until the following year. Suckers growing from diseased branches are commonly very slender and their leaves more or less chlorotic. Such tender twigs, becoming infected at their bases, also often exhibit symptoms of anthocyanescence in their tipmost leaves, the reddish-tipped twigs being very conspicuous in badly diseased trees. As infection continues, more suckers are produced each season, most of which remain on the tree for several years after they are killed. Suckers often grow out in rather compact groups, closely approaching true witches'-brooms, about the bases of killed side branches on the larger limbs and trunks (Plate II, figures 3 and 4). When the tops of small or medium-sized trees are killed entirely the trunk often continues for several seasons to produce abundant crops of suckers at its base.

B. INTERNAL SYMPTOMS

Gross aspects

The characteristic primary leaf lesion originating at the margin of a wound is always preceded by an internal brown discoloration of the vein. This discoloration is usually restricted to the inner core; however, under some conditions it may involve some or all

of the overlying tissues of the vein. In case all of the tissues are involved the discoloration is clearly indicated externally; but when it is restricted to the inner region the browning may be partially or completely obscured by the overlying green tissues. Infected tender leaves, such as those developed on plants growing in the greenhouse in early spring, frequently exhibit venous discoloration externally; harder ones, such as those developed normally in the field, commonly do not do so. In case of artificially inoculated tender leaves, a browning of the severed veins is often the first external indication of infection. The veins may be definitely browned or they may merely appear darker or more opaque by transmitted light than do the nearby uninfected ones. The discoloration, as indicated externally, may involve a large lateral vein and part of the midrib before a yellowing of the mesophyll bordering the wound becomes apparent. In such leaves it is often possible to trace the course of infection from the severed ends of the large lateral veins into the midrib and through the petiole into the stem by externally following the course of venous discoloration (Plate V, figures 1 and 2). In the harder and more robust type of leaf the veins extending out from the necrotic lesion of the blade may be brown or dark discolored for a short distance; but this usually indicates a very small fraction of the extent of internal discoloration. One may easily demonstrate this fact by splitting longitudinally or by making a series of cross-sectional cuts with a sharp blade through a lateral vein or midrib, beginning near a typical primary lesion and working down towards the petiole. Quite frequently the discoloration extends all the way through the larger veins, midrib and petiole, while at the same time the necrotic lesion elsewhere in the blade may involve but a relatively small area at the edge or tip of the leaf.

Nearly always before an infected leaf drops, the brown discoloration already extends into the stem. This is usually indicated by one or more discolored bundle scars on the leaf scar after the leaf is removed. If the stem is split or cut open in a median longitudinal plane through a leaf scar with a brown-discolored central bundle one finds that a narrow brown streak passes into the wood, curves downwards along the pith region and ends blindly (Plate V, figure 2). Similar brown streaks lead into the wood from other discolored bundles. A somewhat later stage of stem discoloration is often exhibited when the bark is removed

from a twig which had previously dropped infected leaves. One or more dark brown streaks, clearly of leaf bundle origin, usually occur in the wood; and they, too, extend downwards from the leaf scar area and end blindly (Plate V, figure 1). In later stages the streak broadens and extends upwards as well as downwards, eventually involving the cambium region and sapwood of the larger branches.

The discoloration of the sapwood and cambium area is one of the most striking symptoms of the Cephalosporium disease of elms. The characteristic form of discoloration is that of diffuse dark brown streaks running parallel with the grain of the wood and readily seen when the bark of infected branches is removed (Plate IV, figures 2 and 3). The streaks may be few, narrow and fairly distinct; or they may have extended periclinally and fused to a greater or less extent, in such cases showing as broad streaks or almost uniformly colored areas. In some instances the discoloration may involve the entire surface of the sapwood for a limited length of branch; but most commonly the browning is more or less restricted to one side of the stem. Frequently one finds all types or degrees of discoloration in a single affected branch. Thus, for some distance below the killed end of the branch, the entire surface of the sapwood may be quite uniformly brown; but farther down the browning may appear as several broad streaks, they in turn finally ending as several smaller and narrower ones; or one large streak or strip may proceed along one side of the branch a considerable distance and then continue as one to several narrower elongated ones into the healthy light-colored wood below. One-sided discoloration in a larger branch may often be traced to one of its affected lateral twigs or to a fork in the branch. It may be noted in passing that the discolored area spreads at a much greater rate with the grain of the wood than against it. This one-sided discoloration and death of the tissues of the cambium and sapwood precedes the development of the elongated sunken cankers previously described.

Instead of stripping the bark from infected stems in order to view the discoloration of the cambium area and sapwood, one may make a clean diagonal cut through the stem, thus exposing other features not exhibited by the former method. In such cuts the discoloration may appear as brown flecks in the outermost ring (or rings) of growth, or more commonly still, as a diffuse browning of the cambium and outer region of the sapwood,

forming a complete ring or crescent-shaped area (Plate IV, figures 4, 5 and 6). The discoloration is most pronounced in the cambium area and outer layers of the sapwood; yet, the color may be diffused throughout the wood in severely infected branches and where the discoloration is restricted to one side there may be a diffusion of color to the center of the stem. The bark covering the discolored cambium and sapwood may also become brown-discolored (Plate IV, figures 4 and 5).

Tissues involved

Microscopic symptoms are all closely associated with the vascular tissues, because the first microscopic symptoms make their appearance in the vessels of the xylem or the parenchyma cells immediately bordering the vessels. This fact holds for leaves just as it does for stems of all ages which may become infected. This discussion of microscopic symptoms is based almost entirely on studies of free-hand sections of field and greenhouse material.

A cross-section through an infected leaf vein which shows a brownish discolored central portion always reveals plugged xylem vessels, or brownish discolored xylem parenchyma, or more frequently both. These symptoms may be restricted to a very small area or they may include the entire vascular strand. In more advanced stages the area of discoloration may include the phloëm area as well as some or most of the outerlying parenchyma surrounding the vascular bundle of the vein. Whether one examines sections through the larger lateral veins, midrib or petiole, the same tissues are involved. Following the discoloration of the vascular tissues, the adjacent tissues of the mesophyll also develop necrotic symptoms.

If one examines a series of free-hand transverse sections of a young stem, beginning immediately above the bundle scars on the leaf scar from which an infected leaf had recently fallen and proceeding downwards, one finds that the discoloration is quite definitely restricted to one, two or all three leaf traces (Plate V). These leaf traces are, of course, the vascular strands which originate in the primary vascular cylinder of the stem and extend upwards and outwards through the secondary xylem, phloëm and cortex into the petiole of the leaf. The bundle scars on the leaf scar represent the distal ends of the traces, the discoloration of which was described above. The discoloration of the leaf trace may extend down along the pith area for an inch or more before

browning includes the neighboring vascular tissues of the primary and secondary xylem. The discolored area finally extends from the embedded leaf trace outwards towards the cambium, commonly forming rather pronounced fan-shaped areas as seen in cross-section (Plate V, figures 3 and 4). The discoloration may or may not include the cambium area by the end of the growing season during which the stem was infected. If not, the browning may extend into the new xylem layer laid down by the cambium the following spring and may later include the cambium. However, in many cases the entire area of xylem and cambium in the vicinity of discolored leaf traces becomes brown and the twig dies by the end of the current season. The tissues exhibiting microscopic symptoms in the wood are quite similar to those of the vascular tissues of the leaf; the vessels, the wood parenchyma surrounding the vessels, near-by ray parenchyma, undifferentiated xylem elements, and cambium are included (Plate VI, figures 1 and 2).

Very soon after the discoloration extends from the wood into the cambium area the bark also becomes discolored. The browning extends outwards from the cambium into the phloëm, and may finally include the pericyclic and cortical parenchyma of young stems, and the phelloderm and phellogen of older ones (Plate VI, figure 6). In older stems with fairly thick bark, however, the first distinct browning may occur in the phellogen and phelloderm areas just beneath lenticels before a browning of the underlying tissues occurs. Such discolored lenticel areas have always been found in bark overlying much discolored sapwood.

Specific microscopic symptoms

1. Chloronemia

This symptom is exhibited in cells of the leaf mesophyll bordering infected veins and chlorophyllous cells of the bark of young stems covering brown-discolored sapwood. Sections through a slightly yellowish area of a typical primary leaf lesion reveal that the chloroplasts are affected. The chlorophyll is destroyed and the plastids become yellowish, smaller and irregular in form, and may finally collect in groups or fuse with one another and form irregular masses. A great contrast exists between the cells in the yellowish area of the lesion and those in near-by green tissue, because the unaffected cells contain an abundance of comparatively large, regular, somewhat flattened or lens-shaped plastids

with a rich green color. Chloronemia is also exhibited in the chlorophyllous cells of the cortex and phelloderm of infected young stems. The symptom may be strikingly demonstrated in a stem which is infected only along one side; the chloroplasts in the cells overlying the browned sapwood may be yellowish, much smaller, irregular, and aggregated, while on the opposite side of the same stem they may appear perfectly green, regular, and in all other respects normal.

2. **Oxychromosis**

The oxidation process which accompanies death of the cells in the leaf lesions, the parenchyma cells closely associated with the vascular system in leaf and stem, and cells of the bark, causes the protoplasmic contents to turn brown. In early stages the protoplast is light or yellowish brown but as the process continues it becomes much darker. Where chloroplasts are present chloronemia precedes oxychromosis. This symptom is commonly very striking in the xylem parenchyma of both leaves and stems. Examinations of cross-sections of an infected midrib often reveal that most, if not all, of the brown discoloration is restricted to the dead protoplasts of the radiating groups or rows of xylem parenchyma. The same is true in the case of infected leaf traces. In stems, wood parenchyma and ray cells which border vessels or groups of vessels are first to exhibit this symptom. However, oxychromosis is also later extended to the protoplasts of undifferentiated xylem elements, cambium, phloëm tissues and all parenchyma cells of the bark. The cell walls, as well as the thick walls of the vessels bordering these browned protoplasts, likewise usually become discolored yellow to brown. Apparently this discoloration is due to absorption of decomposition products of the dead protoplasts.

3. **Abnormal production of tyloses**

According to Gerry (1914), who has made a study of the occurrence and practical significance of tyloses in a number of American woods, tyloses are rarely produced in the American elm. Our observations agree with those of Gerry's in that no tyloses have been found in the twigs and branches which were not diseased. However, in leaves and stems affected with the Cephalosporium disease, tyloses have been observed in the vessels of the veins, midrib, petiole and stem. They are formed in such great abundance in some vessels that the lumina may be almost or com-

pletely occluded (Plate VI, figure 4). At first they are not discolored; many contain nuclei and some develop fairly thick walls. Later, however, their contents may become brown-discolored and their walls partially collapsed. The abnormal production of tyloses is not an exclusive symptom of the Cephalosporium disease, as they also occur in vessels of elms affected with the Dutch elm disease (Schwarz, 1922), Verticillium wilt (Rudolph, 1931) and the Verticillium root disease (Tehon and Jacobs, 1935).

4. **Occlusion of vessels**

The vessels of infected vascular tissues are often partially or entirely occluded in one way or another. Occlusion by tyloses has just been referred to; but the most common and most conspicuous type of plugging is the accumulation of yellowish to dark brown substances in the lumina (Plate VI, figure 5). These brown substances most commonly appear amorphous and gum-like; and not rarely they are bubble-like. The lumina may only be partially occluded by a layer of variable thickness lining the vessel wall or it may be completely plugged. The vessel wall, as well as near-by fibers, may also become brown-discolored. The occlusion of vessels, as is true of the abnormal production of tyloses, is not confined to tissues immediately affected with the Cephalosporium disease, since vessels also become plugged with brownish gum-like substances in stems affected with the Dutch elm disease according to Schwarz (1922) and May (1934) and in roots affected with the Verticillium root disease described by Tehon and Jacobs (1935).

5. **Starch storage interference**

By cutting fairly thin free-hand sections of a diseased stem and then treating them with a solution of iodine (1 gram potassium iodide and 0.3 gram of iodine crystals in 100 c.c. of distilled water), one can determine. the presence or absence of starch. One- to three-year-old diseased and healthy stems were studied and compared for the presence of starch in the various tissues during August. Even without the aid of a lens the contrast between infected and uninfected areas of such sections is very striking; the healthy areas appear blue-black and the infected regions yellowish brown. Starch is absent or greatly reduced in all affected tissues; while in healthy stems or uninfected areas of diseased stems, starch is present in great abundance in the wood parenchyma, ray cells, outer cells of the pith, and all parenchyma

cells of the bark. Wood parenchyma and ray cells bordering vessels bearing hyphae of the pathogene are especially free from starch; but occasionally one finds some starch in the wood parenchyma bordering brownish plugged vessels.

In marked contrast to what has just been described for the tissues affected with the Cephalosporium disease, Buisman (1935) has described and illustrated layers of starch-containing cells formed just beyond areas of xylem affected with the Dutch elm disease. It was observed that the starch in these special layers usually becomes depleted the following spring and apparently normal xylem may be laid down over these tissues. Such starch-containing layers have not been observed in stems affected with the Cephalosporium disease.

C. SIGNS

The presence of minute black pycnidia in the bark of killed twigs, and sometimes in the bark covering cankers, is a sign of the Cephalosporium disease (Plate XVI, figures 1 and 2). These bodies are very small and unless the bark happens to be light-colored or the pycnidia are very numerous they are not readily seen without the aid of a hand lens. Large numbers may be formed on some twigs and very few or none on others. They appear in greatest numbers on the one- and two-year-old twigs and much fewer on older branches. Quite often they can be found in great profusion on leaf scars and in the vicinity of lateral buds of infected twigs. The presence of these small pycnidia, all of which contain multitudes of minute hyaline spores, aids very much in diagnosing the disease.

ETIOLOGY

A. CULTURE STUDIES

Isolation of the pathogene

The fungous pathogene was consistently isolated from both stems and leaves exhibiting symptoms of the Cephalosporium disease. The specimens which were usually selected for stem isolations possessed brown-discolored sapwood and cambium area covered by live or only slightly infected bark. From such twigs and branches small chips of discolored sapwood were removed with a sterile scalpel after carefully stripping back the bark, and then placed on potato dextrose agar slants in culture tubes. In

the course of a few days mycelium grew out of the chips over the agar surface. In the majority of such plantings pure cultures of the pathogene were obtained. By this method, as part of the procedure of diagnosing the disease, more than 500 cultures of *Cephalosporium* have been obtained from nearly 150 diseased trees.

Numerous cultures of the pathogene were also obtained from tissue plantings from diseased leaves. For leaf isolations those bearing typical primary lesions were selected. From each leaf a series of three or four small blocks of the tissue were cut from the blade, the first of which included a portion of the necrotic lesion; and the others were removed at intervals so as to include portions of the main veins or midrib leading from the necrotic lesion toward the petiole. Before each was placed on potato dextrose agar slants in culture tubes, they were surface sterilized in a solution of calcium hypochlorite prepared according to a modification of the method outlined by Wilson (1915) for surface sterilizing seeds. The calcium hypochlorite solution was prepared as follows: 10 grams of commercial chloride of lime (24 per cent Cl) were stirred into 100 cubic centimeters of distilled water and allowed to stand for an hour in a covered beaker; the liquid was then decanted and passed through filter paper. This solution was used immediately or stored in a stoppered bottle; no solution older than several days was used in these studies. For successful sterilization it was found necessary to first dip the blocks of tissue instantaneously into a solution of 95 per cent alcohol to facilitate wetting of the deeper crevices and areas about the leaf hairs before placing them in the hypochlorite solution. A treatment of fifteen to twenty minutes was usually found to be sufficient for surface sterilization. Without washing off the hypochlorite solution each block of tissue was placed in the culture tube by means of sterile tweezers. The fungus often grew out of such preparations in pure culture, especially from blocks of green tissue where infection was restricted to the inner portion of the vein or midrib. Various secondary fungi were usually obtained along with *Cephalosporium* from the more necrotic areas.

Pure cultures for subsequent culture studies and inoculation tests were obtained by cutting out single hyphal tips from two- to four-days-old tissue plantings or from fresh transfers of older cultures. The long sparsely growing hyphae of this fungus in

several-day-old cultures lend themselves admirably to the hyphal tip method of obtaining pure cultures.

Effect of various media on growth

Like many other fungi, *Cephalosporium* does not grow equally well on all types of media. In order to determine what kinds were best suited for various phases of physiological and morphological studies, growth of the organism was compared on ten different types of agar media, three types of solid media and three types of liquid media. In all of these studies only one strain of the pathogene (No. 63-1-3), which originated from a single hyphal tip, was used.

The formulae and the methods used in preparing the various kinds of agar media were as follows:

1. *Potato dextrose.* Broth of 400 g. potatoes boiled in 1,000 c.c. water, 20 g. dextrose, 20 g. agar agar, and water to make total volume 1,000 c.c.
2. *Potato.* Same as for potato dextrose except that dextrose was omitted.
3. *Malt extract.* 30 g. malt extract, 25 g. agar agar, and water to total 1,000 c.c.
4. *Prune.* Juice of 150 g. dried prunes boiled for 30 min. in 1,000 c.c. water, 30 g. agar agar, and water to total 1,000 c.c.
5. *Elm stem dextrose.* Broth from 200 g. chopped elm twigs boiled for 30 min. in 1,000 c.c. water, 20 g. dextrose, 20 g. agar agar, and water to total 1,000 c.c.
6. *Elm stem.* Same as for elm stem dextrose except that dextrose was omitted.
7. *Oat.* Broth of 100 g. rolled oats soaked in 1,000 c.c. water for 1 hour at 60 degrees C., 20 g. agar agar, and water to make total volume 1,000 c.c.
8. *Lima bean.* Broth of 100 g. finely ground lima beans soaked in 1,000 c.c. water for 1 hour at 60 degrees C., 20 g. agar agar and water to make total volume 1,000 c.c.
9. *Corn meal.* Broth of 50 g. of corn meal soaked in 1 liter of water for 1 hour at 60 degrees C., 20 g. agar agar, and water to total 1,000 c.c.
10. *Synthetic nutrient.* 1 g. potassium nitrate, 0.5 g. dibasic potassium phosphate, 0.25 g. magnesium sulphate, 0.20 mg. ferric chloride, 20 g. dextrose, 20 g. agar agar, and 1,000 c.c. of distilled water.

A series of 10 petri dishes for each type of agar was prepared with 10 c.c. of the medium in each dish. A small bit of agar, bearing hyphae from the edge of a giant colony of the pathogene growing on potato dextrose agar, was transferred to the center of each plate. The cultures were all wrapped in paper and allowed to grow at ordinary room temperature (20°–25° C.). At the end of two weeks records were taken of the general characteristics of mycelial growth on the various types of media. These results are summarized in Table I.

The solid media used in these studies included potato cylinders, steamed rice and sterilized elm stems. The potato cylinders and the steamed rice were prepared by the usual methods. The elm stems were prepared as follows: fairly straight healthy twigs one-eighth to one-fourth inch in diameter were collected and cut into pieces three to four inches in length; two pieces of stem and about 3 c.c. of 2 per cent agar were placed in each tube, the agar serving to hold the stems in place and providing a moisture supply; the tubes were plugged with cotton and autoclaved. The general characteristics of the mycelial growth upon these media were briefly as follows:

1. *Potato cylinders.* A cottony growth of mycelium, which was at first white and later grayish to olivaceous brown, soon covered the entire cylinder; the surface of the medium became dark brown.
2. *Steamed rice.* A white to light brown mycelial growth covered all exposed surfaces of the medium; the surface of the medium became light brown while deeper areas became strikingly yellowish.
3. *Elm stem.* A white to light grayish brown mycelium developed on the surface of the agar and up along the surface of the stems, but growth was sparse towards the upper ends.

The three types of liquid media in which mycelial growth was compared included potato dextrose, malt extract and synthetic nutrient. All of these media were prepared according to the formulae outlined above for the corresponding types of agar media except that the agar agar was omitted. Erlenmeyer flasks of 250 c.c. capacity were used for containers; 50 c.c. of the medium were used in each. Cultures were started by drawing a loop of spore suspension through the medium. The rate and amount of mycelial growth were essentially the same on each

TABLE I

EFFECT OF VARIOUS TYPES OF AGAR MEDIA UPON MYCELIAL GROWTH OF THE ELM CEPHALOSPORIUM

Types of Agar Media	Aërial Mycelium			Submerged Myc.		Av. Diam. (2 weeks)
	Color	Amount	Margin	Color	Amount	
Potato dextrose	Grayish olivaceous brown	Abundant, mat medium thick	Grayish, scalloped, smooth	White to brownish	Scant	3.5 cm.
Potato	Grayish green to oliv. brown	Abundant, mat medium thick	Grayish, fimbriate	White to brownish	Very scant	4.0 cm.
Malt extract	Olivaceous brown	Abundant, mat thick	Thick, raised, smooth	Light brown	Fairly scant	1.8 cm.
Prune	Grayish green to oliv. brown	Abundant, mat fairly thick	Grayish, smooth	Brown	Scant	3.0 cm.
Elm stem dextrose	Grayish olivaceous brown	Fairly abundant, mat thick	Smooth	Deep brown	Abundant	5.0 cm.
Elm stem	Gray to grayish brown	Scant, mat thin	Fairly smooth	Light brown	Fairly abundant	4.5 cm.
Oat	Grayish	Scant, mat thin	Fimbriate	Light brown	Fairly scant	5.0 cm.
Lima bean	Grayish	Fairly abundant, mat thin	Thin, somewhat fimbriate	Golden brown	Fairly abundant	5.0 cm.
Corn meal	Grayish	Scant, mat thin	Fimbriate to smooth	Light oliv.	Fairly abundant	4.8 cm.
Synthetic nutrient	Grayish to dark brown	Abundant, mat medium thick	Gray, smooth to fimbriate	Dark brown	Fairly scant	

type of medium. At first numerous small white colonies appeared on the bottom and sides of the flask and on the surface of the liquid. After 3 or 4 weeks the surface was covered by a moderately thick mat of mycelium, which was grayish to olivaceous brown on the upper surface and a darker shade of brown beneath. Considerable mycelial growth also developed beneath the surface of the liquid. The nutrient synthetic and potato dextrose media, which were clear and colorless at the time the cultures were started, always became yellowish as the cultures grew older; this reaction was not noted in the malt extract medium because the liquid was light brown in the beginning.

These tests have revealed what types of media are best suited and what types are not so well suited to the growth of *Cephalosporium;* the particular types of medium suitable for certain physiological and morphological studies have been indicated. The widely used potato dextrose agar was found to be a good medium for general culture work. Mycelial growth on either agar or liquid form of the synthetic nutrient medium was equally good. Since the chemical composition of the synthetic nutrient is known and can be accurately duplicated, this medium, which is a modification of Richards solution similar to that employed by Knudson (1913), was selected for further physiological studies. Indications were also gained as to what types of media were best adapted for development of the several spore forms, a discussion of which has been reserved for the topic of sporulation appearing later in the paper.

Effect of sugar concentration upon mycelial growth.

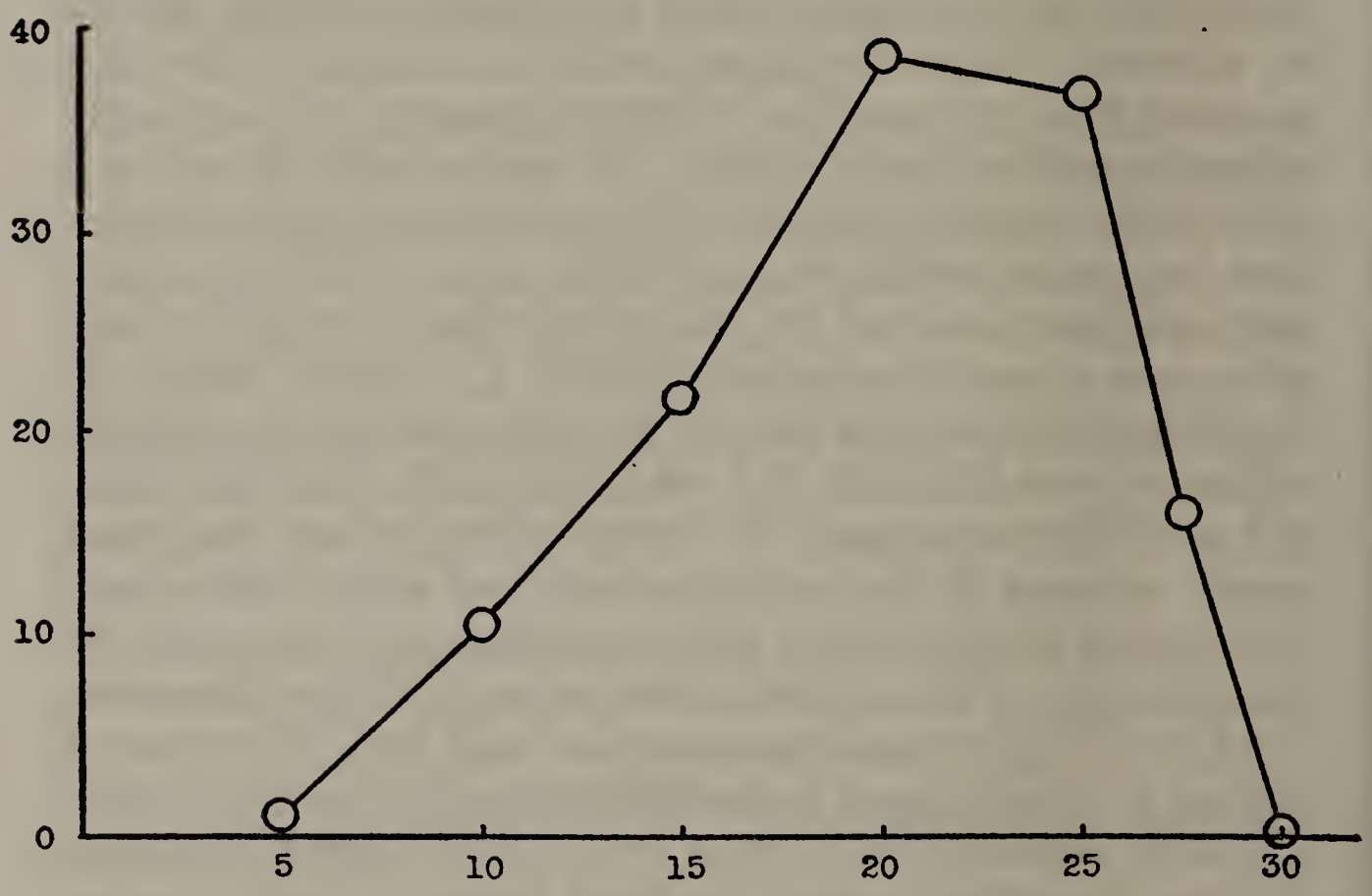
The effect of sugar concentration upon mycelial growth was studied by growing the fungus in synthetic nutrient liquid medium in which the amount of dextrose was varied from 0 to 150 grams per liter. In each series three 250 c.c. Erlenmeyer flasks, each of which contained 50 c.c. of medium, were used for each different concentration. The cultures of each series were all uniformly started by drawing through the medium a loop of spore suspension prepared from a culture which had originated from a single hyphal tip culture (No. 63-1-3). After 4 weeks' growth in a 20° C constant temperature chamber the yields of mycelial growth were determined quantitatively. A method very similar to that used in quantitative chemical analysis, as originated by Knudson (1913), was employed in determining the dry

weight of the mycelium. Numbered Gooch crucibles were chemically cleaned, an asbestos mat was prepared in the bottom of each, and all were dried to constant weight in an oven at 100° C. They were then cooled in a desiccator and carefully weighed in grams to the fourth decimal place. Immediately after weighing they were again placed in the desiccator until used later. From the time the crucibles were placed in the oven to dry, they were always handled with crucible tongs so as not to alter their weights. To each culture flask 50 c.c. of 95 per cent alcohol were added in order to harden the fungus hyphae and facilitate filtering. By the use of suction flasks the fungus mycelium of each culture was separated from the medium by filtering through the previously weighed crucibles; the mycelium was washed with 40 per cent alcohol after the medium had filtered through. They were then dried to constant weight in the 100° C. oven, cooled in a desiccator, and again weighed. By this method the dry weight of each culture was accurately obtained. Using 0, 1, 5, 10, 15, 20, 30, 50, 75, 100 and 150 grams of dextrose per liter, the average yields in milligrams were 8.9, 25.9, 41.7, 44.3, 50.8, 42.9, 44.4, 44.3, 45.8, 44.2 and 43.0, respectively. It is interesting to note that some growth occurred in the complete absence of sugar, while much more growth occurred when sugar was present even in very low concentrations. The fact that there were very little differences found in yields between concentrations of 5 and 150 grams of dextrose per liter appears to be due to some limiting factor other than sugar concentration; it may be that some growth-inhibiting substance or staling product interferes before the sugar concentration becomes a limiting factor. Similar results were obtained when the same medium with the same concentrations of sugar was used in the form of agar in petri dishes. Growth was very sparse on the medium containing no sugar. The optimum concentration for growth was between 10 and 20 grams of dextrose per liter. Development of mycelium was fairly good when as much as 150 or even 200 grams of dextrose per liter were used.

Effect of temperature upon mycelial growth

The effect of temperature upon growth of *Cephalosporium* was studied by growing the fungus in synthetic nutrient liquid medium in a series of constant temperature chambers ranging from 0° to 35° C. The synthetic medium used was the same as that described previously under the heading of the effect of various

media upon growth. For each series three Erlenmeyer flasks, containing 50 c.c. of medium, were used for each temperature condition, except that the 0° and the 35° chambers were eliminated after the first series, since no growth occurred at 0°, 5°, 30° and 35° C. As in the sugar series, each culture was started by drawing through the medium a loop of spore suspension prepared from cultures originating from a single hyphal tip (No. 63–1–3), after which they were immediately placed in their respective



TEXT FIGURE 1. Effect of temperature upon mycelial growth of the elm *Cephalosporium* in liquid nutrient medium.

constant temperature chambers. After four weeks' growth the yields of mycelial growth were determined quantitatively by the use of Gooch crucibles as outlined above. Summarizing results of four series, for each temperature of 5°, 10°, 15°, 20°, 25°, 27.5° and 30° C. the average yields in milligrams were 0.08, 10.3, 21.6, 38.6, 36.8, 16.0 and 0.03, respectively (Text figure 1). Under the conditions of this experiment, the results show that the minimum temperature for growth is in the vicinity of 5° C., the optimum temperature between 20° and 25°, and the maximum at 30°. Cultures which had made no growth while being subjected to a temperature of 0° and 35°, respectively, for 4 weeks were brought

into favorable temperature conditions of the laboratory and allowed to stand for a period of two weeks. At the end of that time abundant mycelial growth occurred in all of the flasks which had been kept at 0° while none occurred in those kept at 35°, indicating that the low temperature merely held back spore germination and mycelial development, while the higher temperature was fatal to the fungus.

In studying the effect of temperature upon mycelial growth of the same pathogene, Goss and Frink (1934) used increase in the diameter of giant colonies as a measure of growth. However, due to the fact that the cultures were thick and compact when grown at some temperatures and thin and wide-spread in others, they pointed out that their measurements did not indicate the effect of temperature upon the total growth. In my studies the method of measuring growth by means of determining the dry weight of the mycelium of each culture completely eliminates such sources of error as encountered by these investigators. Nevertheless, it was interesting to note that the minimum, optimum, and maximum temperatures for growth as determined by this more accurate method were very close to those obtained by Goss and Frink.

Crystal production in cultures

The production of needle-like, lemon-yellow crystals on the upper surface of the mycelial mat and also below the mat in the medium is a striking characteristic of this pathogene (Plate VII). These crystals were alluded to by Verrall and May (1937), but no description of their characteristics was given. When the fungus was grown on a series of agar media it was found that crystals were produced in some types and not in others and where crystals were produced the relative amounts varied with different kinds of media. A characteristic aromatic odor was given off from cultures yielding crystals, and the intensity of the odor closely paralleled the relative amounts of crystals produced.

These yellow crystals were also produced quite abundantly in liquid media of malt extract and synthetic nutrient, each of which contained 20 grams of dextrose per liter. Groups of the needle-like crystals hung down from the bottom of the mycelial mat into the liquid. When such cultures were slightly shaken, multitudes of minute crystals could be seen as their flat sides reflected light, the medium appearing as a sparkling solution. They also developed sparsely in cultures on steamed rice.

Results of preliminary tests indicated that crystals do not develop in media which are not fairly rich in sugar. In order to further investigate this point a series of cultures was grown on synthetic nutrient agar media in which the amount of dextrose was varied; five plates were used for each concentration of 0, 1, 5, 10, 20, 50, 100 and 200 grams of dextrose per liter. After 4 weeks growth, crystals were found in plates containing 20 to 50 grams of dextrose per liter, while none was found in those containing higher or lower concentrations. Whether sugar concentration is directly associated with the chemical reaction of forming the crystals or whether the effect is an indirect one associated with mycelial growth, has not been determined.

Some preliminary studies regarding the chemical nature of the crystals have been made. It has been found that they are readily soluble in 95 per cent alcohol, and by the use of this solvent the crystalline substance may be separated from dried and powdered agar media in which cultures of *Cephalosporium* had grown and produced crystals. By allowing such alcoholic solutions to evaporate in a shallow dish or watch glass the substance recrystallized in the form of flattened needle-like yellow crystals, which by microscopic examination resemble the original crystals in form and color (Plate VII, figure 4). These crystals also gave off the characteristic aromatic odor which is so striking in cultures of the fungus in which the crystals originally form. Preliminary tests conducted by Dr. L. F. Fieser, Professor of Organic Chemistry at Harvard University, showed that the crystalline substance was only slightly soluble in cold water, readily soluble in hot water, and very soluble in ether. Hot aqueous solutions are clear lemon yellow in color and upon the addition of a reducing agent they become colorless.

In studies concerning other fungi references have also been made to the production of characteristic crystal formations in the medium and the liberation of characteristic odors. Pinkerton (1936) described briefly a characteristic type of crystal formation which developed in cultures of *Cephalosporium rubrobrunneum*, a fungus isolated from a human skin lesion. From her brief description it is fairly clear that the crystals produced by the elm *Cephalosporium* are not the same. No statement was made as to whether or not an odor was associated with the crystals. Davidson (1935) has recognized a characteristic odor associated with species of *Endoconidiophora*, a genus of wood-discoloring fungi; the odor

was noted in cultures on agar media as well as in lumber yards. However, no reference was made to any crystalline substance in the medium such as that found to be associated with the odor given off by cultures of the elm pathogene.

SPORULATION

In the early part of my investigation of the Cephalosporium disease serious difficulty was encountered in attempts to identify cultures of the pathogene grown on potato dextrose or malt extract agar because of the scarcity or complete absence of any kinds of spore forms. On these media spore heads, typical for the genus *Cephalosporium*, were rarely found and pycnidial fructifications, characteristic of the Sphaerioidaceae, were almost as rare. Eventually, as a result of various culture tests, satisfactory methods were developed by which large quantities of both spore heads and pycnidia may be produced.

In studies concerning the effect of sugar concentration upon growth the discovery was made that spore heads of the Cephalosporium stage of the pathogene develop in great abundance on the synthetic nutrient agar medium when sugar is completely absent. The mycelium on this sugar-free medium develops sparsely but fairly rapidly, and within 7 days multitudes of spore heads on the ends of short conidiophores develop from aërial hyphae and from those on the surface of the medium. It was interesting to find that spore heads were rarely or not at all produced when as little as 1 gram of dextrose per liter was present in the medium. Likewise, they were rarely or not at all formed on the same medium containing higher concentrations of sugar. Ever since it was found that the Cephalosporium stage developed luxuriantly on this sugar-free synthetic medium, it has been extensively used for obtaining material for morphological studies of this stage of the pathogene, and for obtaining large quantities of spore suspensions for starting series of cultures uniformly and for inoculum in artificial inoculation tests. Much use of this medium has also been made in identifying cultures of the pathogene which fail to sporulate on richer media used in making isolations.

Occasionally pycnidia were formed on potato dextrose or malt extract agar media commonly employed in isolating the pathogene from diseased stems and leaves. But their occurrence was so erratic on these media that difficulty was encountered in attempt-

ing to obtain satisfactory material for studying their development and morphology. Finally, in the sugar concentration studies it was found that pycnidia formed rather abundantly and consistently on the synthetic nutrient agar medium containing 1, 5, 10 or 20 grams of dextrose when the cultures were started with a loop or drop of spore suspension. The medium containing 1 to 5 grams of dextrose per liter proved to be most satisfactory for growing pycnidia for study because the fructifications are not obscured by such a cottony mycelium as develops when higher concentrations of sugar are present. Only a few primordia appeared on the synthetic medium completely lacking sugar, very few pycnidia appeared on concentrations of 50 grams per liter and still fewer when higher concentrations of sugar were present. At the more suitable sugar concentrations pycnidia developed abundantly, beginning in the area formerly occupied by the drop of spore suspension. Compact groups of pycnidia developed near the edge of the area where spores had settled in large numbers, while the central portion was not quite so thickly populated; later, pycnidia usually developed for varying distances beyond the area occupied by the spore suspension. In such cultures pycnidia develop and exude spores in approximately 4 weeks when grown at laboratory temperature (20° to 25° C.). When mycelium is used in place of spore suspension to start cultures pycnidia do not form so readily.

The effect of various media upon the development of pycnidia was also studied. In this test two cultures of the pathogene (No. 1–1–3 and No. 63–1–3), each originating from a single hyphal tip, were grown on the following media: potato dextrose agar, potato agar, malt extract agar, prune agar, elm stem dextrose agar, elm stem agar, oat agar, lima bean agar, corn meal agar, sterilized elm stems, steamed rice and potato plug. Results indicated that pycnidia may develop on a wide variety of media and that abundance of pycnidial development by two different cultures growing under the same conditions may vary greatly. From a standpoint of mycelial color and general cultural characteristics the two cultures appeared to be of the same strain; they were also originally isolated from different trees only several hundred feet apart on the same property, yet many more pycnidia were produced by Culture No. 63–1–3. Pycnidia seemed to develop most consistently on sterilized elm stems; but for development of fructifications for study all of these media proved to be

inferior to the synthetic nutrient agar medium containing 1 to 5 grams of dextrose per liter as previously referred to.

Since pycnidia were found to develop quite consistently on sterilized elm stems, that medium was selected on which to test pycnidial production of a number of hyphal tip cultures. Out of 28 cultures tested 22 produced pycnidia in the bark of the sterilized stems; the abundance produced by the different cultures was various. Cultures producing pycnidia were mostly from Long Island, New York, while others were from Massachusetts, Connecticut, Illinois and Quebec (Canada).

B. MORPHOLOGICAL STUDIES

Mycelium

Among the numerous cultures of *Cephalosporium* isolated from infected trees there was found to exist a rather wide and constant variation in the gross aspects of the mycelium when grown on potato dextrose or malt extract agar medium. The aërial mycelium of all are white or nearly so at the edges of the mat and brownish in the older areas; however, the shade of brown in different strains varied from yellowish, orange, reddish, olivaceous, to grayish. The color of the under surface of the mycelial mat of some cultures is yellow; that of others is brilliant orange, distinctly reddish, or a shade of brown, respectively. These color characteristics appear to be constant; no sectoring or reversion to any other color strain has been observed in any of the numerous cultures studied; single hyphal tip cultures and transfers of these have retained the color characteristics of the originally isolated cultures; repeated isolations from individual trees over a period of three years have always yielded the same color strain; numerous isolants from artificially inoculated trees have also retained the color characters of the original cultures whether reisolations were made by tissue plantings or from pycnidial conidia by the dilution plate method. In only one out of 150 diseased trees from which *Cephalosporium* was isolated, was more than one color strain obtained; in this case two distinct strains were isolated from different branches on the same tree. In all other instances not only was one strain isolated from an individual tree but the same color strain from other infected trees near-by. Indeed, usually no more than one distinct color strain was obtained from any one property or nursery. Besides a constant color variation among the different cultures, there is also a

variation in the texture of the mycelium; some are rather coarse in appearance, while others are more smooth and silky.

Time did not permit a more detailed comparative study of these various strains, so for sake of uniformity only one strain of the pathogene was used almost exclusively in the studies reported in this paper. This selected strain develops a mat of mycelium on potato dextrose agar which is olivaceous brown above, and golden to dark brown beneath; the bottom of the mat has never developed the brilliant yellow, orange, or red color that is so typical for some of the other strains. The hyphae are moderately branched, septate, hyaline at first and light to dark brown later; young hyphae are thin-walled and only slightly constricted at the septa, while older ones may become relatively thick-walled and definitely constricted. When cultures of this strain are grown on favorable media naked spore heads, such as are characteristic for the genus *Cephalosporium*, *Cadophora*, et al., and pycnidia, a type of fructification characteristic for members of the Sphaerioidaceae, are produced. A detailed study has been made of the development and morphology of both types of fructifications and the method of spore formation in each has been determined.

The Cephalosporium stage

Spore heads typical for the genus *Cephalosporium* develop in great abundance on the sugar-free synthetic nutrient agar medium. Conidiophores arise from the aërial hyphae as well as from those growing on the surface of the agar. Conidiophores characteristically occur along slender zigzag or alternately geniculate special branches of the sparsely developed larger aërial hyphae (Plate VIII, figures 1 and 2). The tip of each special branch may terminate as a conidiophore or it may continue to elongate. Occasionally a lateral branch may likewise continue to elongate instead of terminating as a short conidiophore as is usually the case. This alternately geniculate hypha bearing a short conidiophore at each geniculation is very characteristic for this fungus, although the geniculations are not always uniformly alternate and the angle of each geniculation may vary considerably (Plate VIII, figures 1 and 2). There may be only a few conidiophores alternating along such special branches or there may be a dozen or more, but the most common number appears to be five or six. Other conidiophores arise at random directly from the ordinary aërial hyphae. Still others arise from short erect branches originating from hyphae growing from ordinary

elongated hyphal cells; but more frequently they arise from enlarged compact groups of cells resembling stromatic or pycnidial primordia (Plate VIII, figure 3).

Following the conception of Mason (1933) the short branches or terminal portions of hyphae which bear the heads of spores will be referred to as *phialides*, the more or less special hyphae bearing the phialides as *phialophores*, and the spores developed from the phialides as *phialospores*. The term phialide, according to Mason (1933), applies to any "fusiform truncate, fusiformed beaked, or acuminate terminal portion of a hypha, from the apex of which, or within which thin-walled conidia are abstricted". Phialides of the elm pathogene vary from short ampulliform to longer and only slightly tapering, and may or may not be separated from the phialophore by a septum (Plate VIII). The tips of the phialides are quite uniform in diameter, while the lower portions may vary from approximately three times, to only slightly larger than, the diameter of the tips. The terminal portion of each phialide is rounded in the early stages of its development, but after spores have begun to accumulate in heads it is truncate with a more or less open end. The collar at the apex is only slightly or not at all flaring. The phialides as well as the phialophores are hyaline, at least as they occur on the sugar-free agar medium.

The most satisfactory method for studying the structure of phialides and the formation of phialospores has been to stain them in place on the agar surface of petri dish cultures with a one per cent aqueous solution of eosin Y, apply a cover slip, and examine under an oil immersion lens with a magnification of approximately 1400 times. By this method the apical collar or sheath of the phialide is lightly stained and clearly differentiated from the thinner phialospore membrane and protoplasmic contents of both spore and phialide. Neither Amann's acid fuchsin nor Amann's cotton blue was satisfactory. It was found by this procedure that conidia or phialospores are delimited successively from the protoplast in the upper end of the phialide and are extruded one by one through the apical collar. Extrusion of the protoplast usually begins before each spore is completely delimited and becomes abstricted from the inner continuous protoplasmic mass within the phialide sheath a short distance below the apex. Occasionally abstriction takes place farther down before extrusion begins (Plate VIII, figures 2 and 3). These spores are, therefore, endogenously formed phialospores or "endoconi-

dia". The successively formed phialospores are held together by a mucilaginous matrix in the form of sphaerical heads at the tips of the phialides. A head of average size contains 30 to 50 spores. In a nearly saturated atmosphere the diameters of 300 spore heads in a number of 7-day-old petri dish cultures were measured at random, and they were found to range from 4.9 to 14.8 microns with an average of 8.5 microns in diameter. The mucilaginous substance in which the spores are imbedded is quite hygroscopic; so the diameter of the spore head would undoubtedly vary with the moisture content of its immediate environment. When the moisture content is high the spores appear to be very loosely arranged in the watery matrix; while under dry conditions they are held in a compact agglutinated mass. It is quite likely that this hygroscopic substance offers protection to the very thin-membraned conidia under adverse moisture conditions, and makes dissemination in free water possible under conditions favorable for dissemination and germination.

The phialospores are one-celled, hyaline, ellipsoidal to cylindrical, sometimes slightly curved, and range from 1.1 to 2.3 by 3.0 to 6.0 microns with an average of 1.6 by 4.4 microns (based on measurements of 300 spores). These spores all germinate readily in drops of water or on agar media. In several hours the volume of the spore increases two or three times, becomes broadly ellipsoidal to sphaerical, the contents become vacuolate and granular, and within 12 to 24 hours a germ tube (which soon becomes septate and branched) is developed (Plate VIII, figures 4 and 5). Usually only one germ tube is formed from each spore but occasionally two or three have been observed.

The structure of phialides and the nature of phialospore formation in the elm *Cephalosporium* has not heretofore been described. But the same method of endogenous spore formation has been recently described in detail by Pinkerton (1936) in species of *Cephalosporium* isolated from human skin lesions, by Andrus and Harter (1933), Davidson (1935), Gwynne-Vaughan and Broadhead (1936) and others in the conidial stage of *Ceratostomella fimbriata* (Ell. and Hals.) Elliott and related species, by Lagerberg, Lundberg, and Melin (1927), Melin and Nannfeldt (1934) and Davidson (1935) in members of the genus *Cadophora*. Indeed, similar conidia are apparently not infrequent throughout the Ascomycetes and Fungi Imperfecti. The classical example of endoconidial formation appears to be that in *Thielavia basi-*

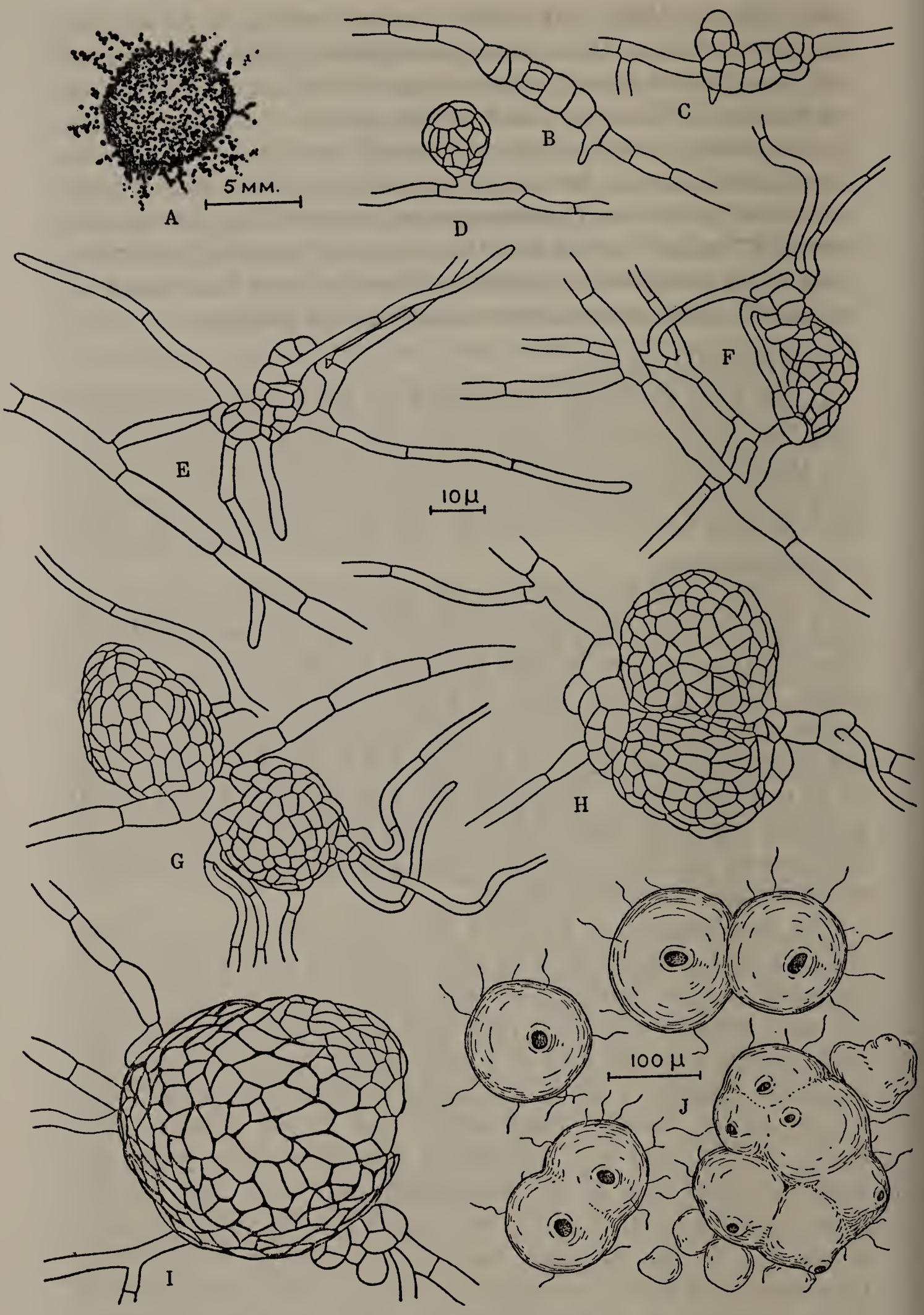
cola. Gilbert (1909) states that the endoconidia of *T. basicola* are produced from the copious protoplasm within the terminal cell, that this cell opens by bursting or dissolution of the tip, that the conidia are slowly pushed out by growth of the protoplasm in the swollen basal portion of the cell, and that new conidia form continuously in the rear of those being ejected. The conidial formation in the elm *Cephalosporium* is essentially the same as that in *T. basicola* except that the spores are delimited nearer the apex of the phialide and ejection appears to be at least aided by increased enlargement of the vacuoles in the phialide.

The pycnidial stage

It has been definitely established by pure culture technique that the hyphomycetous spore-head type and the sphaerioidaceous pycnidial type of fructifications are developed by the same fungus; spores from spore heads and spores from pycnidia produce mycelia with identical characters and each produces both types of fructifications.

Pycnidia occur in nature on the surface of leaf scars, in the bark of one- and two-year-old killed twigs and occasionally in the bark over cankers in older branches of the suscept. They are small, black, globose, with or without short necks, ostiolate, solitary to compactly grouped, and early erumpent. The diameter of pycnidia was found to average 138.6 microns with a range of 90 to 210 microns. Measurements were made of a total of 500 pycnidia which included current season and overwintered fructifications on naturally infected twigs, and some which developed on artificially inoculated plants.

The development of pycnidia has been studied in petri dish cultures on synthetic nutrient agar as well as in the bark of the naturally and artificially inoculated suscept. (Text figure 2, A.) On agar medium pycnidial primordia or stromatic initials arise from several adjacent cells of a main hypha or from similar cells in a short branch. Cells of the hypha enlarge, repeatedly divide in all planes, become thick-walled and brown-colored (Text figure 2, B, C and D). Sometimes hyphal branches grow out from the outer cells of the primordium and these may anastomose with one another and other hyphae near-by (Text figure 2, E and F). As this stromatic mass of cells enlarges it commonly develops into a single pycnidium or it may develop two to several distinct lobes each of which may eventually grow into a pycnid-



TEXT FIGURE 2

ium (Text figure 2, G, H, and I). When more than one pycnidium develops from a single primordium or stromatic initial their walls usually become quite fused where they grow in contact with one another (Text figure 2, J). Whether pycnidia are single or grouped each develops a definite ostiole.

The development of pycnidia in the bark of elm twigs has been studied in transverse and longitudinal sections of naturally and artificially inoculated stems. The material was killed and fixed in weak chromo-acetic solution, softened in 50 per cent hydrofluoric acid, imbedded in paraffin, sectioned 10 microns thick, and stained with safranin and light green. Free-hand sections of fresh material stained with Amann's cotton blue, Amann's acid fuchsin, or aqueous solutions of eosin Y were also helpful in this study. Stromatic or pycnidial initials were found to originate in the phellogen or phelloderm tissue beneath the cork layer from hyphal cells in much the same manner as they do on agar medium. At first the stromatic mass consists of solid pseudoparenchymatous tissue of light brownish cells (Plate IX, figures 1 and 2). As it enlarges by division of cells throughout its compact mass the outer layers become brown and thick-walled, while the inner region usually consists of thinner-walled hyaline or only lightly colored cells. Eventually a small cavity appears in the central area which very early contains multitudes of small conidia (Plate IX, figure 3). The pycnidial stroma may or may not break through the bark before the pycnidial cavity begins to form; but the majority of those encountered in this study did not become erumpent until after the cavity was well formed (Plate IX, figures 3, 4 and 5). From the numerous sections studied, indications are that only one pycnidium is usually formed from each stromatic or pycnidial primordium. Pycnidia were often found

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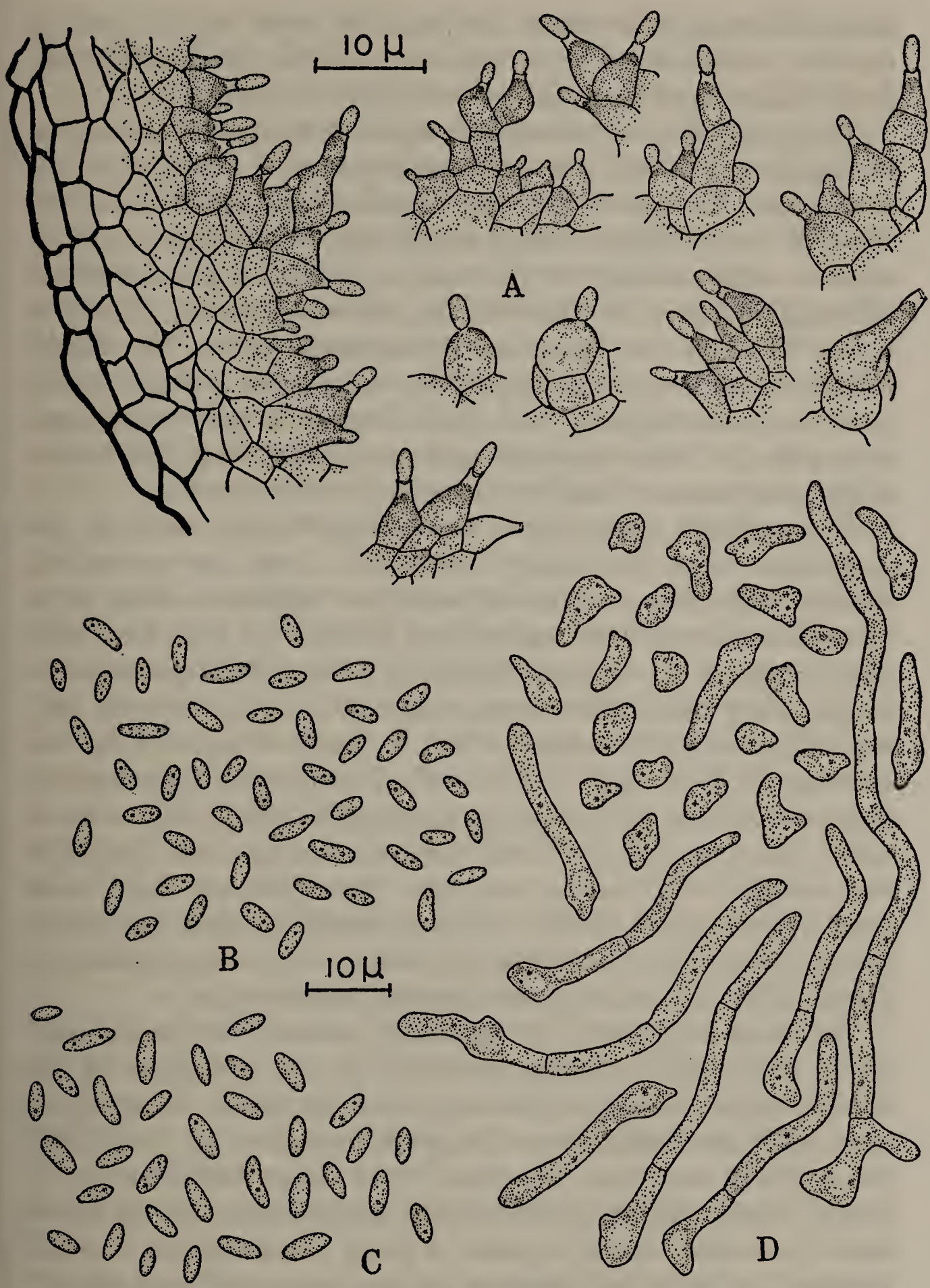
TEXT FIGURE 2. Origin and development of pycnidia of the elm *Cephalosporium* on agar medium in petri dishes. A. Surface view of a culture showing pycnidia developed in and near an area previously occupied by a drop of spore suspension. B. Early stage of primordium developed from a main hypha. C. A somewhat later stage than that in B. D. Primordium originating from a short lateral branch. E. Primordium showing its rather thick wall and recently formed ostiole. F. Anastomosing hyphal branches originating from a pycnidial primordium. G. Two pycnidial fundaments originating in close proximity from the same hypha. H. A two-lobed primordium. I. A single immature pycnidium previous to development of ostiole. J. Sketches of mature pycnidia illustrating varying degrees of fusion with one another.

to be compactly grouped and their walls fused, but nearly all seemed to originate from separate initials when observed in their early stages of development (Plate IX). In a few instances, however, more extensive stromata have been observed in which there might have developed more than one pycnidium. The internal cavity of the pycnidium continues to enlarge and ultimately an ostiole is formed (Plate IX, figures 4 and 5). The wall of the pycnidium is quite thick when young and as it grows older the wall becomes thinner, especially in the lower part; it varies in thickness from two or three to six or eight cells (Plate IX, figures 4, 5 and 6).

From an early stage a definite sporogenous layer, consisting of multitudes of rounded to flask-shaped phialides, lines the bottom and side walls of the pycnidium (Text figure 3, A). These pycnidial phialides are essentially like those formed directly from the hyphae in the so-called Cephalosporium stage. In fact, the shorter phialides developed from stromatic initials on the surface of the sugar-free agar medium as illustrated in Plate VIII, figure 3, appear to be morphological types intermediate between the elongated ones developed on the aërial hyphae (Plate VIII, figure 2) and those developed in the pycnidium (Text figure 3, A). The pycnidial phialides may be sessile or they may appear singly or grouped on short phialophores. They are usually conical or flask-shaped with a truncate apex or neck through which the small phialospores are successively ejected. Yet some have been observed which are merely rounded cells lacking the neck-like projection and from which phialospores are ejected through a pore in the top or apical area of each. The phialospores are delimited from the protoplast in the apical end of the phialide and appear to be abstricted from the inner protoplasmic mass within the sheath or apical collar. Ejection of the spore begins before delimitation and abstriction is complete. Whether these phialospores are true endoconidia may be a matter of opinion since abstriction takes place so near the apex of the phialide. In favor of the view that they are endoconidia is the fact that an empty sheath or collar may be clearly seen at the apex. As more and more spores are formed the vacuolar contents of the phialide appear to increase as the protoplasmic mass decreases. In several instances phialides devoid of protoplasm with open apical ends were observed, indicating that the number of spores produced by any one phialide is limited. It appears that new phialides are con-



TEXT FIGURE 3. Details of the pycnidial stage of the elm *Cephalosporium*. A. Portion of a median section of a mature pycnidium showing sporogenous layer, and numerous phialides showing variation in structure and manner of spore formation. B. Pycnidial conidia. C. Spore-head conidia — for comparison with pycnidial conidia in B. D. Germinating pycnidial conidia.

stantly forming and taking the place of those which cease to function; this seems to be the explanation for the gradual decrease in the thickness of the pycnidial wall as it grows older.

The pycnidial cavity becomes completely filled with multitudes of conidia imbedded in a mucilaginous matrix. In a saturated atmosphere the spores and their mucilaginous matrix ooze out through the ostiole and collect at the end of the short neck or apex of the pycnidium in the form of a pinkish glossy globule (Plate XVI, figure 3). Upon drying, the mucilaginous globule of spores becomes an agglutinated hardened pellet and firmly adheres to the top of the pycnidium, which upon return of moist conditions absorbs water and again becomes a watery mucilaginous globule. Since the mucilaginous substance is highly hygroscopic the spores are readily disseminated in free water.

The pycnidial phialospores are essentially like those of the spore-head type previously described. They are one-celled, hyaline, ellipsoidal to cylindric, sometimes slightly curved, often contain one or two oil droplets, and average 1.2 by 3.2 microns with a range of 1.1 to 1.5 by 2.3 to 5.3 microns (300 spores measured). They differ from those produced in heads in being consistently somewhat smaller, which is clearly illustrated by the drawings in Text figure 3, B and C. The pycnidial phialospores germinate readily in water or on agar media. They swell to 2 or 3 times their normal size, their contents become more granular and vacuolate and one to two germ tubes grow out in 12 to 24 hours (Text figure 3, B and D). On a suitable medium the germinating spores develop a cottony mycelium in all respects like that produced by the spores of the Cephalosporium stage.

The stages in pycnidial development, details of the sporogenous layer, and method of spore formation in the pycnidium of the elm pathogene have not heretofore been described. In disagreement with the description of the pycnidium given by Verrall and May (1937), the writer has found that the pycnidial stroma is nearly always subphellar instead of subepidermal, the pycnidia often have short necks in place of being non-papillate, a sporogenous layer of conidiophores is present instead of being absent, and conidia are definitely formed in phialides instead of being formed histogenically. However, measurements of the mature pycnidium and the pycnidial conidia reported in this paper compare quite favorably with those reported by Verrall and May (1937); and those of the spore head conidia compare favorably

with those reported by May (1931), Goss and Frink (1934), and Verrall and May (1937).

Like many other Fungi Imperfecti this fungus is very difficult to classify according to our present inadequate systems of classification. On the basis that the conidia were borne in mucilaginous heads at the ends of conidiophores it was first tentatively placed in the form-genus *Cephalosporium* by May (1931). As established by Corda (1839), the genus *Cephalosporium* is restricted to forms which have hyaline or bright-colored hyphae. Under certain conditions the elm pathogene may be considered a good *Cephalosporium*, as for example, when the organism is grown on the sugar-free synthetic nutrient medium the hyphae remain hyaline and an abundance of mucilaginous spore heads is formed. However, when the same organism is grown on a medium containing sugar the hyphae are hyaline only when they are very young and gradually become distinctly brown. Therefore, since the hyphae are not hyaline or bright-colored it could not be a *Cephalosporium*. When one considers that the hyphae are brown, and phialospores are formed endogenously, the fungus appears to be very closely related to species of *Cadophora* Lag. and Melin, a form-genus of the subdivision Chalareae of the Dematiaceae. This genus was established by Lagerberg, Lundberg and Melin (1928) on a single species of wood-staining organism; but other species of wood-staining fungi have recently been included in the genus by Melin and Nannfeldt (1934), and Davidson (1935). The hyphomycetous stage of the elm pathogene seems to be most closely related to such species as *C. obscura* Nannf. and *C. repens* Davidson. Since it has been definitely proved that the organism also produces a distinct pycnidial stage in the bark of the suscept as well as in artificial culture it can hardly be classified in either genus referred to above. On the basis of the pycnidial stage Verrall and May (1937) have recently named the pathogene *Dothiorella ulmi*, n. sp. This is perhaps as good a position for the organism as any other at the present time. Nevertheless, there is some doubt in my mind as to whether or not the basal stroma described by these authors is definite and common enough to warrant its being placed in that genus. It has been my experience that in the great majority of instances pycnidia are solitary or merely fused together as a result of mutual contact. Since the term stroma is so loosely defined in systematic treatments of the Fungi Imperfecti and is so variable in even those forms which unquestionably

develop a stroma, it seems to be a very unsatisfactory character for separating even form-genera. Also before this pathogene can be satisfactorily classified still more information concerning the morphology and the development of the organism is desirable. In this connection the discovery of a perfect stage would aid greatly. Thus far no ascomycetous form has been obtained in overwintered material or by crossing various strains in artificial culture. The size and method of formation of conidia in both hyphomycetous and sphaerioidaceous stages of the elm pathogene resemble microconidia or spermatia of many Ascomycetes. The fact that they germinate so readily would not preclude the possibility of their being microconidia or spermatia of an ascomycetous type according to Dodge (1932), who has clearly demonstrated that in a number of instances microconidia may either function as spermatia or as ordinary conidia which germinate readily and develop mycelia.

Since there is still some doubt in my mind as to the proper position of this fungus, I have chosen to refer to the pathogene in this paper as the "elm *Cephalosporium*" and to the disease as the "Cephalosporium disease of elms", because of the fact that the name *Cephalosporium* has come to be so commonly associated with this disease.

C. INOCULATION STUDIES

Pathogenicity tests

In order to investigate the relative virulence of several selected strains of the elm *Cephalosporium* and to establish further proof of pathogenicity, artificial inoculation tests were conducted in the greenhouse on seedlings of *Ulmus americana*. These tests constitute two separate experiments, the seedlings of the first being inoculated at the base of the stem, and those of the second at the tip.

Experiment I.

Three sets of 60 seedlings each were used as suscepts in this experiment. The first two consisted of approximately one-year-old-plants grown from seed in the greenhouse and the third consisted of two-year-old plants obtained from an Ohio nursery. Those of each set were selected as uniformly as possible for size and general health, and each plant was well established in a separate pot at the time of inoculation.

Five cultures of the elm *Cephalosporium* were selected as sources of inoculum, each of which was derived from a single hyphal tip culture. Those selected represented at least three, and possibly four, different strains so far as their cultural characteristics were concerned. The original source and characteristic of these selected cultures are briefly presented in Table II. Cultures No. 1–1–3 and No. 63–1–3 were representatives of the same strain. Both were isolated from different near-by trees on the same property and in nature appeared to be of a very virulent strain. Culture No. 63–1–3 was the same as that used in the culture studies previously described in this paper and is also a representative of the same strain described in detail under the heading of morphological studies. The Nebraska culture, No. 793–1–1, was kindly sent to me by Dr. R. W. Goss in June 1934. This culture was originally isolated from a tree in Lincoln, Nebraska, in 1932, and was the culture employed in inoculation tests reported in the bulletin by Goss and Frink (1934). The culture used in my studies was derived from a single hyphal tip of the original culture received from Dr. Goss. Culture No. 118–1–1 was quite similar in appearance to that of the Nebraska culture, and No. 222–1–1 varied considerably from all of the others. Each of these strains was grown on potato dextrose agar slants in test tubes and the cultures were approximately two weeks old at the time each set was inoculated. The inoculum for each plant inoculated consisted of a bit of the actively growing mycelium along with a small portion of its agar medium.

Ten plants of each set were inoculated with each of the selected strains of the pathogene. The method of inoculation consisted in making a small cut with a sterile scalpel through the bark, extending downwards for about one-eighth to one-fourth of an inch between the bark and the wood at the base of the stem one to two inches above the soil surface. The inoculum was immediately introduced into the open wound with a sterile inoculation needle, and a small piece of absorbent cotton was wrapped about the stem over the wound which was then saturated with water and bound with raffia. Also 10 plants of each series were similarly wounded but instead of being inoculated with the pathogene, sterile potato dextrose agar was inserted in the wound, and then they were similarly bound with water-saturated cotton and raffia to serve as checks. All plants of each series were placed in the saturated atmosphere of a moist chamber for 48 hours after inocu-

TABLE II

CULTURE CHARACTERISTICS OF THE ELM CEPHALOSPORIUM ISOLANTS USED IN THE PATHOGENICITY TESTS

Culture No.	Original Source	Date Isol.	Color of Myc. Mat on P. D. A. Medium		Color of P. D. A. in Old Cultures	Pycnidia On Ster. Elm Stems
			Above	Below		
1–1–3	Westbury, New York	1934	Grayish to olive-brown	Dark golden brown	Yellowish	Few
63–1–3	Westbury, New York	1934	Grayish to olive-brown	Dark golden brown	Yellowish	Abundant
222–1–1	Glen Cove, New York	1934	Grayish brown to reddish brown	Bright orange to reddish brown	None	Fairly abundant
793–1–1	Lincoln, Nebraska	1932	Buff or grayish to brown	Dark reddish brown	Pinkish to rose-red	None
118–1–1	Danielson, Conn.	1934	Grayish to cinnamon-brown	Dark brown to blackish	Pinkish	Fairly abundant

lation. The first set was inoculated November 9, 1934, after most of the leaves had fallen. After the inoculated plants were taken from the moist chamber they were kept in a cool greenhouse until December 5, at which time all were placed in an unheated pit to overwinter. On February 14, 1935, they were brought into the heated greenhouse. Plants of the second set were of the same age as those of the first, were overwintered in the same place, and were brought into the heated greenhouse at the same time. However, this set was not inoculated until March 12, 1935, after buds had broken and many new leaves had developed. The two-year-old plants of the third set were also brought out of their winter quarters on February 14, 1935, and were inoculated at the same time as those of the second set after dormancy had broken and new leaves were rapidly growing. Each of the 180 plants involved was numbered and notes of the symptoms and general health of each were recorded at frequent intervals throughout the season; thus a complete case history was obtained for each plant. However, only a generalized summary of the various symptoms expressed by the infected plants in the experiment as a whole can be briefly outlined in this presentation.

The first symptom of infection to occur was that of retardation or complete failure of buds to open in a number of the inoculated suscepts of the first set in early spring following inoculation. Some of the plants were killed before buds began to swell, others were killed very soon after buds had swollen and begun to elongate, while still others were not killed until after retarded buds gave rise to short etiolated stems and small chlorotic leaves. These early symptoms appeared at about the time plants of the second and third sets were being inoculated. The first symptoms of infection in the last two sets appeared five weeks after inoculation as a yellow mottling and slight drooping of the leaves of the lower branches on the side of the stem bearing the inoculation wound.

Abnormal stimulation of lateral bud development and consequent production of suckers was a symptom exhibited by a number of infected plants in all three sets (Plate X, figure 1). Commonly the young tender leaves of these suckers and other tender branches of diseased plants exhibited symptoms of anthocyanescence, which is due to the abnormal production of a red pigment in the leaf mesophyll.

A yellowing or yellow mottling of leaves was a common occur-

rence in those plants inoculated in the spring as well as in those inoculated in the fall, especially when they were not affected too severely before their leaves were well developed (Plate X, figure 2). Mottling usually appeared first between the main veins at the base of the leaf and advanced outwards along one or both sides of the midrib; in others the yellowing first appeared at the tips and edges of the leaf. In either case such leaves often became quite yellowish at the edges and in the areas between the main veins, leaving the veins and mesophyll immediately bordering them quite green as in the upper leaves of the plant illustrated in Plate X, figure 2. These yellowed leaves may or may not develop brown necrotic areas before they finally drop. Infected plants exhibiting such foliage stand out in marked contrast to healthy green uninoculated check plants.

Perhaps the most striking symptom exhibited by infected plants was that of sudden blighting of leaves. Leaves became only lightly mottled or not at all, suddenly wilted or drooped, became dry, curled and brown, and clung to the twig indefinitely. In some instances these symptoms occurred in scattered leaves far away from the point of inoculation, and in others all of the leaves of a branch and even the entire top suddenly became blighted at the same time (Plate X, figure 3). When small tender twigs were blighted their tips usually curved into the characteristic shape of "shepherds' crooks" and often retained the dried and curled terminal leaf after all others had fallen. Defoliation of yellowed and blighted leaves continually occurred throughout the season.

Sunken, elongated cankers developed upwards from the point of inoculation in the larger stems of some of the two-year-old plants, and the bark of affected young stems of some became discolored dark brown to black. All plants which exhibited external symptoms of infection, and in some of those inoculated which did not, possessed a marked brown discoloration in the cambium and sapwood area. The discoloration always originated at the inoculation wound and commonly occurred along one side of the stem in the form of diffuse streaks.

The symptoms exhibited by these artificially inoculated plants simulate very closely those exhibited by young twigs and branches in the field which have become infected through their bases, or quite some distance from their tips, through previously infected side branches or through infected main branches from which they may be growing.

By the first week in June a number of inoculated plants had been completely killed and others were badly diseased in all three sets, and some, especially those of the first set, were killed much earlier. By the end of the first season following inoculation one-half to two-thirds of the plants inoculated with each strain had been killed, except for those inoculated with the Nebraska strain (No. 793-1-1). The results of the experiment as recorded near the end of the first season on September 24, 1935, are briefly summarized in Table III. All of the plants inoculated with the Nebraska strain were not only alive at the end of the season, but no symptoms of infection were exhibited at any time. Since similar results were obtained in other experiments, further remarks regarding this strain will be reserved for a later part of the discussion.

TABLE III

DATA SUMMARIZING RESULTS OF AN EXPERIMENT IN WHICH THE PATHOGENICITY OF SEVERAL STRAINS OF THE ELM CEPHALOSPORIUM WAS TESTED BY INOCULATING SEEDLINGS OF ULMUS AMERICANA THROUGH BASAL STEM-WOUNDS

Inoculum	First Set		Second Set		Third Set		Total	
	Plants inoc.	No. killed	Plants inoc.	No. killed	Plants inoc.	No. killed	Plants inoc.	No. killed
63–1–3	10	7	10	5	10	8	30	20
1–1–3	10	1	10	6	10	5	30	12
222–1–1	10	3	10	6	10	6	30	15
793–1–1	10	0	10	0	10	0	30	0
118–1–1	10	6	10	6	10	7	30	19
Checks	10	0	10	0	10	0	30	0

Pycnidial fructifications of the pathogene developed in large numbers in the killed stems of many of the inoculated plants. They were found in scattered groups on the twigs and topmost branches as well as on the main stem near the point of inoculation where they occurred most commonly. The pathogene was consistently reisolated from killed and diseased plants of each set from the discolored sapwood of the stem, even from the topmost branches. All cultures obtained were identical in color and general appearance with the original strain used as inoculum.

The plants of the third set, which had been inoculated with cultures No. 63–1–3 and No. 222–1–1 but did not exhibit external symptoms of disease by the end of the first season, were found to possess brown discolored streaks in the sapwood leading upwards from the inoculation wound. All of these plants were retained throughout the next season, but again no external symptoms of infection occurred. All were dissected at the end of the second season to determine the extent of internal discoloration. In most of them the discoloration extended upwards from the inoculation wound for less than two inches but in a few the streak extended upwards for six to twelve inches. In all cases the discoloration had not extended into the current season's sapwood but was restricted to that of preceding years' growth. Tissue plantings were made from the discolored sapwood of several of these plants in order to determine whether or not the pathogene could be reisolated. From one of these in which a brown streak near the pith area extended up the stem from the inoculation wound ten to twelve inches, three out of four plantings yielded pure cultures of the pathogene; all others yielded negative results.

Throughout the course of the experiment all uninoculated check plants remained perfectly healthy, exhibiting no external symptoms and no internal discoloration. Tissue plantings were made from the sapwood of several of the check plants as well as from some of those which had been inoculated with the Nebraska strain, but cultures of the pathogene were obtained from none.

Experiment II.

This experiment was quite similar to the one just described except that tips of young twigs were inoculated instead of the basal regions of older stems. Fifty seedlings of *Ulmus americana* which were two years old and of uniform size were brought out of winter quarters into the greenhouse on February 15, and were inoculated on April 12. At the time of inoculation leaves were well developed and the young shoots were rapidly elongating. Cultures used as inoculum in this experiment were the same as those used in Experiment I except that No. 222–1–1 was omitted. Two of the topmost young twigs of the current season's growth on each of 10 plants were inoculated with each of the selected strains of the elm *Cephalosporium*. The third or fourth leaf from the tip of the stem to be inoculated was removed and by first making a cut through the bark immediately in front of the axil-

lary bud, the bark including the bud and leaf scar was stripped back along one side of the stem exposing the surface of the sapwood for an area of one-eighth to one-quarter of an inch long. A bit of mycelium along with a small portion of the medium was immediately placed in the wound with a sterile inoculation needle. The bark was then brought back over the inoculum and the wound was bound with water-saturated absorbent cotton and raffia. As checks against those inoculated, 10 other plants were similarly treated, except that sterile potato dextrose agar was substituted for mycelial inoculum. After inoculation all plants were placed in a moist chamber for 48 hours and then were allowed to continue growth on the greenhouse bench. All were numbered and notes of the condition of each were recorded at frequent intervals throughout the season, so that a rather complete record of the symptoms for each infected plant was obtained. However, as in the preceding discussion of Experiment I, only a generalized summary of the symptoms expressed by the infected plants as a whole will be outlined here.

Symptoms of infection began to appear in many plants of this experiment in four weeks after inoculation. Most frequently the first indication of infection appeared in the leaf just above the inoculation wound on the same side of the stem; the leaf drooped, became yellow mottled and soon fell, or suddenly wilted, became dry, curled, and greenish brown, and for a time remained in place on the stem (Plate XI, figure 1). Later, other leaves on the same side of the stem and finally, those on the other side exhibited similar symptoms. In some plants all of the leaves beyond the point of inoculation either became yellow mottled and drooped or all suddenly wilted, and became dry and brown, before they finally fell. Quite frequently these symptoms were preceded by the symptom of anthocyanescence in the youngest leaves of the inoculated twig, and in others the first symptom to appear was the sudden wilting and drying of the tipmost leaf instead of the leaf near the point of inoculation. In either case drooping and blighting of the leaves beyond the inoculation wound occurred, and in most cases included leaves below the wound as the season advanced.

Very often the midrib and lateral veins of yellow mottled or wilted leaves became discolored brown, the discoloration first appearing at the base of the midrib and proceeding towards the tip and into the lateral veins.

Twigs from which leaves had fallen frequently exhibited dark brown, slightly sunken, necrotic flecks or spots in the greenish tender bark. Such spots usually grew larger and coalesced, causing rather extensive areas of the bark of the infected stem to become darkly discolored and shriveled.

TABLE IV

DATA SUMMARIZING RESULTS OF AN EXPERIMENT IN WHICH THE PATHOGENICITY OF SEVERAL STRAINS OF THE ELM CEPHALOSPORIUM WAS TESTED BY INOCULATING SEEDLINGS OF ULMUS AMERICANA THROUGH WOUNDS IN YOUNG STEM TIPS

Inoculum	Number Inoculated		Number Infected	
	Plants	Stem Tips	Plants	Stem Tips
63–1–3	10	20	10	19
1–1–3	10	20	8	12
118–1–1	10	20	9	19
793–1–1	10	20	0	0
Checks	10	20	0	0

By September 24 of the first season following inoculation most of the infected plants exhibited marked symptoms of die-back. The extent of die-back varied from none, in such cases where only several leaves near the infection court had fallen, to the other extreme where both inoculated twigs had been completely killed and where symptoms of die-back included the upper portion of the main stem. But in most instances only 8 to 12 inches of the inoculated twigs were involved (Plate XI, figures 2, 3 and 4). None of the plants was completely killed during the first season, but 60 to 95 per cent of all twigs inoculated with each of the different strains of *Cephalosporium*, except those inoculated with the Nebraska strain, exhibited positive symptoms of infection. As in Experiment I, no infection occurred in any of the plants inoculated with culture No. 793–1–1 or in any of the uninoculated check plants. A summary of the results of this experiment as recorded near the end of the first season, September 24, 1935, is given in Table IV.

Pycnidia of the pathogene occurred quite abundantly in the bark of many of the infected twigs, especially within several inches

of the infection court (Plate XI, figure 5). The pathogene was reisolated from the diffuse, brown-streaked sapwood as well as from pycnidial conidia from a number of the infected twigs. All attempts to isolate the organism from check plants yielded negative results.

All of the plants of this experiment were retained throughout the second season after inoculation. They were overwintered in an unheated pit and brought back into the heated greenhouse early the following spring. By the end of the second season dieback progressed very little in most of the plants, but several of them were entirely killed and in one which had died back to within 4 inches of the soil surface there developed an abundance of suckers at its base. The pathogene was readily isolated from the tissue of these plants and in one instance a culture was obtained from the crown an inch below the soil surface.

The symptoms and signs exhibited by the plants in this experiment were almost identical with those expressed in the field by young twigs which became infected through the leaf traces from previously wounded and infected leaves.

The combined results of both of these experiments demonstrate that the elm *Cephalosporium* is pathogenic to American elm seedlings. Symptoms of the artificially inoculated plants resembled in nearly all details those exhibited by infected trees in the field, the pathogene was consistently reisolated from plants exhibiting those symptoms, and pycnidial fructifications of the pathogene were formed in many of the infected and killed stems. The results corroborate those of earlier though less extensive experiments of Verrall (1930), May (1931), and Goss and Frink (1934).

Since not more than 50 inoculations were made with each of the selected strains of *Cephalosporium*, the results may not be considered entirely conclusive; but certainly they strongly indicate that there may be considerable variation in the relative virulence or degree of pathogenicity among different strains of the pathogene and even among different cultures of apparently the same strain. In these tests culture No. 63-1-3 proved to be the most virulent, but No. 118-1-1 was almost as virulent. It was quite surprising to find such a wide difference in the percentage of infection between those inoculated with No. 63-1-3 and No. 1-1-3. As indicated previously in this discussion (Table II), both of these cultures represented the same strain and appeared equally vig-

orous in mycelial growth. It is noteworthy that before they were selected for this test it was recognized that No. 63-1-3 always produced a much greater abundance of pycnidia as well as a greater abundance of the characteristic lemon-yellow crystals in suitable media than were produced by culture No. 1-1-3. Why it was that no infection occurred in any of the fifty inoculations made with the Nebraska culture, No. 793-1-1, is not known. In previous experiments by Goss and Frink (1934) the culture was proved to be quite pathogenic, but their tests were conducted almost two years before the culture was forwarded to me. On potato dextrose agar transfers of the culture produced as vigorous mycelial growth as any of the cultures which I had recently isolated. According to these tests its pathogenicity has been lost; but since no further investigations have been made, no assured explanation can be offered. The other four cultures were isolated during the summer of 1934, and the conditions throughout both experiments have been kept as uniformly as possible in testing pathogenicity of each strain. Therefore, the percentage of infection yielded by each of these strains should be a comparable test of relative virulence.

Throughout these tests as a whole, as well as in groups of plants inoculated with a single strain of the pathogene, there was noted a marked difference in the relative susceptibility or tolerance to the pathogene among the different plants inoculated. In those inoculated at the base of the stem, some were entirely killed by the last week of May, some were not killed until much later in the season, a few were only partially killed by September, one was never known to produce external symptoms even though the pathogene was isolated from its stem two years after inoculation, and in others no symptoms at all were exhibited and the pathogene could not be reisolated from their inoculated stems. Similar results were noted in Experiment II, in that nearly the entire tops of a few plants were killed by the end of the first season, just the inoculated twigs were killed back from their tips in others, in a few only several leaves dropped near the infection court with no die-back resulting, and in others no infection whatsoever was indicated (Plate XI, figures 2, 3 and 4). These observations concerning the relative susceptibility of artificially inoculated seedlings of American elm are quite comparable to field observations; some infected trees seem to be killed in a relatively short time

while others seem to be capable of tolerating infection for many years.

LEAF WOUNDS AS INFECTION COURTS.

Results of preliminary tests conducted in the field during the first season of this investigation indicated that the pathogene gained entrance to the suscept through wounds in leaves following artificial inoculation. Since no leaf infections, either as a result of artificial or natural inoculations, had been reported by previous investigators, a series of intensive artificial inoculation tests was conducted on leaves of elm seedlings in the greenhouse.

Experiment I. Wounded versus unwounded leaves.

The purpose of this experiment was to determine whether or not infection might occur in unwounded as well as wounded leaves following artificial inoculation. One-year-old seedlings of *Ulmus americana* were used as suscepts and two forms of inoculum, mycelium and spore suspension of culture No. 63–1–3, were used throughout the experiment. The suscepts were divided into three sets of 30 seedlings each, the first set being inoculated on February 22, the second on March 16, and the third set on April 13, 1935. The mycelial inoculum consisted of a small bit of mycelium along with a little of the medium from an actively growing culture two weeks old on potato dextrose agar. The spore suspension was prepared from mature exuding pycnidia in 20 to 25 c.c. of sterile water. The pycnidia used in its preparation developed in cultures four weeks old on potato dextrose agar in 500 c.c. Erlenmeyer flasks.

In each series five leaves on each of 15 plants were wounded with a sterile scalpel by severing one of the large lateral veins transversely about half way between midrib and leaf margin (Plate XII). The other 15 plants were uninjured. Five of the wounded and five of the unwounded plants were inoculated with mycelium of the pathogene by placing some inoculum immediately over each of the 5 leaf wounds of each wounded plant and on each of 5 leaves of each unwounded plant. Likewise, a drop of spore suspension was placed over each leaf wound of 5 other wounded seedlings and on 5 leaves of each of 5 other seedlings which were not wounded. The remaining 5 wounded and 5 unwounded plants were retained as controls or checks against those inoculated. Immediately after inoculation all were placed in a moist chamber for 48 hours and then were allowed to continue de-

velopment on the greenhouse bench. Each of the three sets was similarly treated. Each plant as well as each leaf inoculated on each plant was numbered and records were taken of symptoms on individual leaves as they occurred and as the disease progressed at frequent intervals throughout the season.

The earliest symptoms appeared in the wounded inoculated plants in 10 to 14 days after inoculation. Usually the first symptom observed was a slight yellowing of the blade near the inoculated wound (Plate XII, figure 1). The yellow zone usually developed most rapidly towards the midrib but in many instances the area soon extended from margin to midrib in the wound area. It commonly progressed most rapidly along the midrib towards the petiole in the half bearing the wound, but later involved the blade on both sides of the midrib. In some instances a yellow mottling occurred more or less generally over the entire leaf. Such infected leaves became more yellowish, wilted and soon dropped, or developed a conspicuous brown necrotic area in the vicinity of the wound which was surrounded by a yellowish border, finally became dry and curled upwards at its tip and margin, and frequently clung to the stem indefinitely (Plate XII, figure 2). Early symptoms in these tender leaves also included a browning of the veins beginning at the edge of the inoculated wound and proceeding downwards into the midrib, through the petiole, and into the stem through the leaf traces. Such browning of the severed veins frequently preceded the yellowing of the mesophyll in the vicinity of the wound (Plate XII, figures 4 and 5). In some of the wound-inoculated seedlings all five leaves exhibited symptoms of primary infection, in others only one to several became infected, and in still others none of the wounded leaves exhibited any symptoms of infection (Plate XIII, figures 1 and 2). When no symptoms were exhibited in a wounded leaf in 4 to 5 weeks after inoculation it usually never became infected. The primarily infected leaves began to drop in 2 to 4 weeks after the first symptoms were observed. Shortly after the infected leaves dropped, the leaf scar became brown and sunken, the axillary bud became shriveled and often abscissed, and beginning near the leaf scar sunken elongated brown to blackish flecks or streaks appeared in the bark (Plate XIII, figure 3). Following defoliation of the primarily infected leaves the other leaves on the stem above and below the inoculated ones exhibited symptoms of infection. They first became yellowish mottled at the base and

up along the midrib. Mottling was usually accompanied by a drooping of the blade and a browning of the midrib and lateral veins beginning at the petiole. They finally became yellow mottled all over, wilted and dropped; or in some instances they became dry, curled upwards from their margins and tips, and turned brown before they finally fell. The leaves above were nearly always first to show these symptoms but later the lower leaves were also included. The bark of the killed tips became blackish brown and shriveled, resembling in all respects those symptoms which follow infection of wounded leaves naturally inoculated in the field (Plate XIII, figure 4; and Plate III, figure 3). Some of the infected seedlings were killed and had developed multitudes of pycnidia in the bark of the dead stems in three months after inoculation (Plate XIII, figure 5). Some were not entirely killed until much later in the summer, while others were killed back from their tips but possessed many suckers at the base, and still others dropped a few primarily infected leaves but the stem was not killed. The sapwood of infected stems always exhibited the typical brown discoloration, which was readily traced into the stem through leaf traces from the primarily infected leaves.

The pathogene was consistently reisolated from primarily infected leaves exhibiting the above described symptoms (Plate XIII, figure 2), as well as from defoliated stems following infection through wound-inoculated leaves (Plate XIII, figure 4). Portions of the infected leaf blade were cut out, beginning near the wound and removing blocks at intervals towards the petiole so as to include portions of the severed vein and midrib which in many cases were discolored brown. Such blocks of leaf tissue were washed in sterile water several times and finally were surface sterilized in a solution of calcium hypochlorite as previously described. Within several days after planting on potato dextrose agar in petri dishes pure cultures of the pathogene grew out of each block of infected tissue (Plate XIII, figure 6). The hyphae first grew out of the cut ends of veins and in instances where infection was more or less restricted to the central core of the midrib a fan-like colony of the fungus grew out over the agar from each cut end of the vein. Similarly, pure cultures of the pathogene grew out of the surface-sterilized infected stems forming fan-shaped colonies about the open ends and leaf scar areas

over the surface of the potato dextrose agar in petri dishes (Plate XIII, figure 7).

A summary of the results of this experiment is given in Table V. These data show that no infection whatsoever occurred in any of the inoculated unwounded seedlings or in any of the wounded and unwounded checks, while 22 out of 30 wound-inoculated plants exhibited positive symptoms of infection. All infected plants with their symptoms of yellowing and browning of the leaves, defoliation, and dying back of the stem tips stood out in marked contrast to those of the healthy green check plants (Plate XIII). No significant difference was noted in the symptoms or relative number of infections occurring in the wound-inoculated seedlings whether mycelium or spore suspension was used as inoculum. From the results of this experiment one may conclude, therefore, that wounds in leaves serve as ideal infection courts or openings through which the elm *Cephalosporium* may gain entrance to the suscept.

Experiment II. Kinds of leaf wounds.

In the preceding experiment only one type of wound was employed, namely, that in which one of the large lateral veins was severed transversely about half way between the margin and midrib in the central area of the blade. Since infection occurred quite readily when such leaves were inoculated, it was thought advisable to compare as infection courts this type of wound with one in which only the small netted veins between the larger laterals were severed. The latter was made by cutting with a sterile scalpel a slit parallel with and equidistant between two of the main lateral veins about half way between the margin and midrib in the central area of the leaf. Both types of wounds were essentially of the same length, about one-quarter of an inch long, and were inflicted in comparable areas of the leaf blade. For simplicity of reference, the type of wound in which a large lateral vein was severed transversely will be referred to as the "perpendicular" type, and the other which was parallel with the large laterals will be referred to as the "parallel" type. These two types of wounds are illustrated in Plate XII, figures 1 and 3. Plants used in this test consisted of 30 two-year-old seedlings of *Ulmus americana*, each of which had been established in individual pots. They were brought into the greenhouse from winter quarters on February 15, and were wounded and immediately

TABLE V

DATA SUMMARIZING RESULTS OF AN EXPERIMENT IN WHICH WOUNDED AND UNWOUNDED LEAVES OF ULMUS AMERICANA WERE INOCULATED WITH TWO FORMS OF INOCULUM OF THE ELM CEPHALOSPORIUM

		Inoculated						Checks	
		Wounded			Unwounded			Wounded	Unwounded
		Myc.	Spore susp.	Total	Myc.	Spore susp.	Total		
First Set	Plants inf.	4	4	8 (80%)	0	0	0	0	0
	Leaves inf.	15	15	30 (60%)	0	0	0	0	0
Second Set	Plants inf.	3	2	5 (50%)	0	0	0	0	0
	Leaves inf.	5	3	8 (16%)	0	0	0	0	0
Third Set	Plants inf.	5	4	9 (90%)	0	0	0	0	0
	Leaves inf.	16	9	25 (50%)	0	0	0	0	0
All Sets Combined	Plants inf.	12	10	22 (73%)	0	0	0	0	0
	Leaves inf.	36	27	63 (42%)	0	0	0	0	0

inoculated on April 16. At the time of inoculation leaves were well developed, but the young shoots had not ceased elongating. "Perpendicular" wounds were inflicted in leaves of 15 of the plants and "parallel" wounds in leaves of the remaining 15 seedlings. A single wound was inflicted in each of 10 leaves per plant, which involved 5 consecutive ones on each of two main topmost branches. As inoculum two types of spore suspension, both of which were derived from the single hyphal tip culture No. 63–1–3, were employed; the one was prepared from conidia of the "spore head" or Cephalosporium stage which were developed in cultures four weeks old on sugar-free synthetic nutrient agar, and the other from pycnidial conidia which were developed in cultures of the same age on malt extract agar. Of the plants bearing "perpendicular" wounds, five each were inoculated with each of the two types of spore suspension and five others were retained as checks. Likewise, of those bearing "parallel" wounds five each were inoculated with each of the two types of inoculum and the remaining five were retained as checks against those inoculated. Immediately after the inoculum was applied all of the plants were placed in a moist chamber for 96 hours. They were then removed and placed on the bench in the greenhouse, each plant was numbered, and records were kept of the developments of each throughout the season.

Symptoms of primary infection began to appear in both types of wounded leaves in two weeks after inoculation as a yellowing of the mesophyll in the vicinity of the wound and a discoloration of the severed veins. These early symptoms, as well as those which followed, resembled in all respects those of the wound-inoculated leaves of the preceding experiment which were described in detail. Even though symptoms began to appear in both types of wounded leaves at about the same time, symptoms of infection in those with the "perpendicular" type of wound progressed much more rapidly and infection occurred in three times as many of the inoculated leaves as in those with the "parallel" type of wound. The results of this experiment as recorded six weeks after inoculation are summarized in Table VI. By the end of September a number of these plants exhibited typical symptoms of die-back of the branches which previously bore the inoculated leaves (Plate XIV). Such symptoms appeared among plants inoculated with either type of inoculum and among those with either type of leaf wound. However, in some instances even

though most of the inoculated leaves became infected and the discoloration extended into the stem no die-back resulted by the end of the first season. This apparent difference in susceptibility of the different plants to the pathogene was very marked in this experiment, even in cases where the same source of inoculum was used and the same type of wound had been inflicted in the leaves (Plate XIV, figures 2, 3 and 4). From the results of this experiment one may definitely conclude that leaf wounds in which only the smaller veins are severed, as well as those in which the larger veins are involved, may serve as infection courts for the elm *Cephalosporium;* but those with the larger severed veins are the more favorable. It has also been demonstrated that either conidia of the Cephalosporium stage or those of the pycnidial stage of the pathogene may serve as inoculum.

TABLE VI

DATA SUMMARIZING RESULTS OF AN EXPERIMENT IN WHICH TWO TYPES OF WOUNDS IN LEAVES WERE COMPARED AS INFECTION COURTS FOR THE ELM CEPHALOSPORIUM

		Inoculated			Checks
		Pyc. spores	Ceph. spores	Total	
Perpendicular Wounds (Across veins)	Plants inf.	5	5	10 (100%)	0
	Leaves inf.	48	47	95 (95%)	0
Parallel Wounds (Between veins)	Plants inf.	4	4	8 (80%)	0
	Leaves inf.	23	9	32 (32%)	0
Data of Both Types Combined	Plants inf.	9	9	18 (90%)	0
	Leaves inf.	71	56	127 (61%)	0

Experiment III. Age of leaf wounds.

In the preceding experiments leaves were inoculated immediately after wounding. The present experiment has been outlined to determine whether or not infection might also take place through older wounds. Twenty two-year-old seedlings of American elm were used as suscepts and mycelium of culture No. 63–1–3

was used as inoculum. Ten leaves per plant were wounded by severing one of the larger lateral veins, described as the "perpendicular" type of wound in the preceding experiment; five plants were wounded 4 days before inoculation, five others 2 days before, the same number one day before, and five others were inoculated immediately after wounding. All were inoculated at the same time, April 20, 1935, with mycelium from the same potato dextrose agar culture. Immediately after inoculation all were placed in a moist chamber for 72 hours, and after that time were placed on the greenhouse bench where they remained throughout the season.

Summarizing the results of this experiment, for leaves bearing wounds 0, 1, 2 and 4 days old, the percentage of infection was 46, 40, 18 and 16, respectively. Even though each wound-age was represented by only 50 leaves, the results demonstrated that infection may occur in leaves bearing wounds at least 4 days old, but that the percentage of infection was significantly greater in those inoculated immediately after wounding.

Experiment IV. Susceptibility of different species of *Ulmus*.

It has been definitely proved in preceding tests that *Ulmus americana* is susceptible to the elm *Cephalosporium* with either wounds in leaves or wounds in stems as infection courts. In this experiment five other species of the genus *Ulmus* have been investigated. The species included were as follows: *U. pumila* L., *U. foliacea* Gilib., *U. japonica* Sarg., *U. glabra* Huds. and *U. Thomasi* Sarg. All were approximately one year old at the time of inoculation. Five leaves per plant were wounded by severing one of the large lateral veins and in all instances mycelium of culture No. 63–1–3 was used as inoculum. The number of plants of each species inoculated and the number used as checks, as well as a summary of the results, are presented in Table VII.

Since the number of plants of each species varied and since in most cases there were so few involved, their relative susceptibility is not indicated by these results; but it was definitely proved that *U. pumila* and *U. japonica* are susceptible to the elm *Cephalosporium*. Typical symptoms of infection, in all respects comparable to those previously described for wound-inoculated leaves of *U. americana*, were exhibited by the infected leaves of both of these species (Plate XV, figures 1 and 2). Symptoms of primary leaf infection were followed in succession by those of secondary

leaf infection, defoliation, development of sunken dark brown areas in the bark, and eventually by typical symptoms of die-back (Plate XV, figures 2 and 3). The pathogene was reisolated from both infected leaves and stems in a number of instances; and in the case of *U. pumila* the hyphae of the pathogene were followed from the infection court through the severed lateral vein into the midrib, through the petiole into the leaf traces, and from the leaf traces into the surrounding vessels of the wood by microscopic examination of a series of stained sections.

TABLE VII

SUSCEPTIBILITY OF ULMUS SPECIES TO THE ELM CEPHALOSPORIUM WITH LEAF WOUNDS AS INFECTION COURTS

		Inoculated		Checks	
		Number inoculated	Number infected	Number involved	Number infected
U. pumila	Plants	40	21	40	0
	Leaves	200	67		
U. japonica	Plants	10	2	10	0
	Leaves	50	5		
U. foliacea	Plants	10	5	10	0
	Leaves	50	11		
U. glabra	Plants	3	1	2	0
	Leaves	15	3		
U. Thomasi	Plants	5	0	5	0
	Leaves	25	0		

In *U. foliacea* and *U. glabra* some of the inoculated leaves developed yellowish lesions and brownish veins near the wounds. The leaf traces became discolored in several instances but the disease progressed no farther. Since only a few plants of these two species were used in the test, and the pathogene was not reisolated, one may only say that they, too, are probably susceptible. No symptoms whatsoever developed in any plant of *U. Thomasi*, even though they were inoculated twice during the season. The results indicate that this species is not susceptible to the elm

Cephalosporium, yet since these negative results are based on so few plants one cannot draw the definite conclusion that the species is wholly resistant.

D. LIFE HISTORY STUDIES

Sources of inoculum

It has been proved by artificial inoculation tests that mycelium, conidia of the spore head stage and pycnidial conidia of the elm *Cephalosporium* may all serve as inoculum. In nature killed twigs and cankers appear to be the chief sources of inoculum in which both mycelium and pycnidia overwinter in viable condition. These overwintered twigs commonly bear multitudes of the small black pycnidia which project through the surface of the bark singly or in groups, or they may appear to be almost superficial as is frequently the case when the thin cork layer under which they developed peels off (Plate XVI, figure 1). In some twigs and cankers they may be very sparsely developed and in others very profusely. In one instance, with the aid of a lens, 634 of these small black bodies were counted in an area of one-quarter inch square; thus more than 10,000 may be present on the surface of a single piece of twig less than one-quarter inch in diameter and two inches in length. They are globose to flask-shaped and may be non-papillate or possess short necks (Plate XVI, figure 2). Each contains myriads of minute conidia imbedded in a slimy or mucilaginous matrix which under moist conditions ooze out through the ostiole and collect at the apex in the form of a pinkish glossy droplet (Plate XVI, figure 3). Hundreds of colonies of the pathogene have been obtained from such spore droplets by the dilution plate method. When dry overwintered pycnidia were collected in early spring and placed in a moist chamber droplets of spores were exuded in 12 to 24 hours. Within 48 hours aërial hyphae grew out from these droplets. They also grew out from the necks and other exposed surfaces of the pycnidia and from crevices in the bark. On these hyphae multitudes of naked spore heads of the Cephalosporium stage developed. It seems probable, therefore, that under prolonged periods of moisture, as is quite common during the spring months, these spore head conidia, as well as those exuded from the overwintered pycnidia, may provide inoculum for new infections. The more or less superficial pycnidia are quite easily dislodged either in pieces or as a whole from the weathered dead twigs. Since they

are so small they too may possibly serve as inoculum; under favorable conditions bits of the wall as well as their conidial contents give rise to vegetative hyphae.

No detailed study has been made to determine what agent or agents are responsible for transporting the inoculum from the source to the infection court; but circumstantial evidence indicates that the inoculum may be dispersed in drops of water and carried by the wind during rains to uninfected parts of the same or near-by trees. Many primary lesions have frequently been observed on branches which were formerly healthy near previously killed twigs that were bearing an abundance of pycnidia. Similarly, seedling trees beneath large severely diseased ones have been observed to be infected in varying degrees, the nearest ones rather severely, others beyond only slightly and those farther away not at all. Such observations tend to indicate that the inoculum was splashed down from the source above to the infection courts below. This view is plausible when one considers that it is under moist conditions that the slimy droplets of conidia ooze from the pycnidia and that free water readily disperses the spore mass. The possibility of wind alone acting as an agent of inoculation seems to be rather remote, since the conidia of both Cephalosporium and pycnidial stages are firmly held together by the mucilaginous matrix during moist periods and are even more firmly held during dry periods when the matrix becomes gummy and finally hard.

The sticky nature of the mucilaginous spore masses suggests the possibility that insects which might come in contact with any one of the many exuding pycnidia over the surface of the bark may be carriers of the elm *Cephalosporium*. Such insects which inhabit the trees in great numbers during the spring and early summer months, for example, canker worms or elm leaf beetles, may very well be responsible for distributing the inoculum over the same and near-by trees. Another indication that insects may act as agents of inoculation has been the common observation that pycnidia are hollowed out or partially eaten or entirely cleaned from the twig by mycetophagous insects. In passing it may also be pointed out that higher animals such as birds, squirrels, and even man may also be responsible for spore dissemination of this pathogene as they too must frequently come in contact with the spore-bearing bodies during periods which favor spore exudation.

INFECTION COURTS

Artificial inoculation tests have demonstrated that leaf wounds as well as stem wounds provide suitable infection courts through which the elm *Cephalosporium* may gain entrance to the suscept. Results of preliminary leaf inoculations suggested the possibility that since insect feeding wounds were so abundant in leaves of the elm they may serve as ideal infection courts. Following this lead, intensive field investigations were conducted and it was soon established beyond any doubt that insect feeding wounds in leaves are the most common infection courts in nature. In the vicinity in which this study was made the fall canker worm (larvae of *Alsophila pometaria* Harr.) was responsible for most of the leaf injuries which served as abundant infection courts for this elm pathogene. The leaves were injured during May and early June and the first symptoms of leaf infection were observed in the summer of 1935 on June 15 and in the season of 1936 on June 25 (Plate XVI, figure 4). In artificial inoculation tests two to three weeks usually elapsed from the time of inoculation to the appearance of symptoms. Based on these results indications are that early inoculations occurred in the field during the height of the canker worm feeding season. Symptoms became very conspicuous during the last of June and July and were present in abundance throughout the season in trees bearing sources of inoculum and to a lesser extent in others near-by. In all details the symptoms of these naturally wounded and inoculated leaves in the field were the same as those of artificially wounded and inoculated leaves in the greenhouse. Vascular discoloration of the veins, midrib and petiole accompanying these lesions extended into the sapwood of the stem through the leaf traces. This discolored streak in the sapwood extended downwards for a short distance and gradually ended blindly, thus indicating that the causal agent entered from the leaf and not from the stem into the leaf. By culture studies involving nearly 500 tissue plantings from over 100 specimens, it was definitely shown that the elm *Cephalosporium* is associated with such leaf lesions. In 75 per cent of all specimens cultured the pathogene was obtained from the leaf blade in the vicinity of the lesion, from discolored veins, midrib and petiole associated with the lesion, and from short discolored streaks leading into the stem from the infected leaf, but not from the stem a short distance below or above the leaf attachment. In many instances the pathogene was isolated near the

limited yellow area of the lesion and from the larger veins and midrib a short distance away, while plantings made from the lower portion of the blade, petiole and stem to which it was attached all remained sterile. Thus, the results of culture studies, as well as those in which the progressive stages of the symptoms have been followed, indicate that the pathogene causing the lesion near leaf wounds gains entrance from the outside.

Even though injuries made by the fall canker worm were found to be the most common infection courts for the elm *Cephalosporium* during this investigation, many other insects may create equally suitable infection courts in leaves, such as the spring canker worm (*Paleacrita vernata* Peck), spiny elm caterpillar (*Hamadryas antiopa* Linn.), elm leaf beetle (*Galerucella luteola* Müll.), white-marked tussock moth (*Hemerocampa leucostigma* S. and A.), and many others. Still other insects, such as larvae of the leopard moth (*Zeuzera pyrina* Linn.), small European bark beetle (*Scolytus multistriatus* Marsh), elm twig girdler (*Oberea tripunctata* Swed.), buffalo tree hopper (*Ceresa bubalus* Fabr.) and others, may create possible infection courts in stems. However, in only one instance was such a stem wound found to be the center of an infection of the Cephalosporium disease. One branch about two inches in diameter at the base suddenly developed yellowing and blighting of the leaves. Further examination revealed that a leopard moth wound was present about three feet from the base. In the sapwood marked brown streaks extended downwards to within 6 inches of the base and upwards into the smaller twigs, the streaks becoming fewer and narrower as the distance from the wound increased. Cultures of the elm *Cephalosporium* were obtained from the discolored sapwood from points distributed along the branch from about one foot below the wound to the smaller branches above. It is believed that the leopard moth tunnel must have been the infection court because the appearance of external symptoms of yellowing and wilting occurred generally over the entire branch. The indications were that infection had occurred below and not at the tips. Moreover, the internal discoloration was most extensive in the vicinity of the wound.

In addition to wounds inflicted by insects, as has been indicated by the results of artificial inoculation tests, any open wound in a stem or leaf, such as hail injury in leaves, wind-broken twigs,

unprotected pruning wounds and the like, may all provide possible infection courts for the elm *Cephalosporium*.

COURSE OF INFECTION

Since leaf wounds are the most common infection courts of the elm *Cephalosporium* in nature, a leaf wound will be taken as the starting point in discussing the course of infection. After the inoculum arrives at the infection court an incubation period of a week or more precedes infection and subsequent expression of symptoms. In the wound the spores germinate and the hyphae produced grow into the xylem vessels of the severed veins. The progressive discoloration of the vascular cylinder of the veins, midrib, petiole, leaf traces and xylem of the stem, is a general indication of the path taken by the hyphae and the accompanying infection (Plate XVI, figures 4 and 5; Plate V). The course of infection has been followed in detail in series of both free-hand and microtome sections of veins, midribs, petioles and stems, including the leaf traces, some of which were collected in the field and a number of those which had been artificially inoculated with pure cultures of the pathogene in the greenhouse. Microtome sections were prepared from material showing symptoms of various degrees of infection by killing and fixing in weak chromo-acetic solution, softening in hydrofluoric acid solution, imbedding in paraffin, sectioning with a rotary microtome, and staining with safranin and light green. Safranin and Haidenhain's iron-alum haematoxylin were also used as stains in some of the preparations; but the combination of safranin and light green proved to be the most satisfactory in following the path of hyphal growth, especially through necrotic tissue outside vessel lumina. Free-hand sections of similar material which were examined before and after staining with either cotton blue, acid fuchsin, or Giemsa stain in Amann's fluid helpfully collaborated the serially cut and permanently mounted preparations. Both cotton blue and acid fuchsin were useful in staining the fungus hyphae in the vessels and the Giemsa stain was quite useful in demonstrating affected parenchyma cells. Giemsa stained the protoplasm greenish to blue-black when infected and not at all or only lightly bluish or pinkish when healthy. This stain was readily taken up by all cell contents exhibiting any symptoms of oxychromosis whether the discoloration was only slightly yellowish or dark brown. The yellowish to brown gum-like substance which often plugs the

lumina of vessels was also similarly stained. This stain, therefore, was quite an aid in detecting infected areas which otherwise might have been overlooked.

Hyphae of the pathogene were traced into the smaller as well as the larger lateral veins from the wound. They were traced downwards into the midrib from one vessel element into another through terminal perforations and occasionally from one vessel into another through pits. In all cases relatively few hyphae were present in the vessels of infected veins and midrib, the intensity and scope of infection appearing quite out of proportion to the presence of so few strands of hyphae (Plate XVI, figure 5). Frequently all hyphae entering the midrib from a severed lateral vein occupied a few vessels only on one side and these often eventually proceeded into the stem through but one of the lateral leaf traces. In other instances they were more equally distributed throughout the vascular strand before the petiole was reached and consequently the hyphae occupied all three leaf traces as they grew into the stem from the leaf. Growth proceeded through the leaf traces downwards into the primary xylem near the pith region of the stem. Eventually by growing through pits from one vessel into another the area of infection in cross-section gradually increased, and growth of the hyphae proceeded upwards as well as downwards in the vessels of the wood as they grew from one element into another through terminal perforations. As in the vessels of leaves, hyphae were not found in all vessels of an infected area in a stem and they were often very few in each vessel (Plate VI, figure 3). On the other hand sometimes they were quite abundant, branched, irregularly coiled, and occupied much of the vessel lumen (Text figure 4). In both stems and leaves the hyphae were restricted to the lumina of vessels in the beginning and as long thereafter as the surrounding tissue remained alive. Numerous free-hand sections examined before and after staining with Giemsa stain in Amann's fluid have indicated that infection occurs a short distance in advance of the invading hyphae. Such was noted in veins and midribs of infected leaves as well as in the stems. By examining free-hand sections of infected stems and making cultures from contiguous portions of the same it was consistently demonstrated that tyloses, discoloration of xylem parenchyma, and the occasional plugging of vessels occurred in advance of the hyphal growth as the area of infection proceeded from the tip towards the base of the stem. The wood parenchyma

TEXT FIGURE 4. Hyphae of the elm *Cephalosporium* in xylem vessels of *Ulmus americana* as seen in transverse section of an infected stem.

and ray cells bordering vessels containing hyphae were always found to be affected even though the fungus had not come into contact with them. Eventually not only were living cells which bordered the infected vessel killed but others were affected some distance away, resulting in the appearance of tyloses and the brownish gum-like material in the near-by vessels. After the living cells of the wood were killed, the hyphae of the pathogene grew out into the ray cells and wood parenchyma and even into the pith parenchyma of the young twig. As the newly formed vessels and their surrounding tissues became infected the cambium was ultimately killed and the area of infection soon included the phloëm and the other living tissues of the bark. These tissues, like those of the xylem, were occupied with the hyphae of the pathogene soon after being killed, penetration occurring while the tissues were still moist and succulent. By the time the pathogene entered the young stem through leaf wounds and finally penetrated the wood from the leaf traces outwards into the bark, the epidermis of the stem was no longer alive and in many instances was sloughed off and a cork layer of several cells in thickness had taken its place. Hyphae invading the tissues of the infected bark became quite concentrated in the phelloderm and phellogen tissues but they never entered the phellem. Pycnidial primordia commonly originated in great numbers from the hyphal cells beneath the cork layer, eventually developing into mature erumpent pycnidia (Plates XI and XVI). On such killed twig tips, which had become infected through leaves injured by canker-worms in the spring, sporulating pycnidia in the bark were first observed in the middle of August. It is quite probable, however, that those which often form on the exposed leaf scars may develop and sporulate somewhat earlier than those in the bark. Spores produced by these bodies may very well initiate secondary cycles of infection in injured leaves and result in the establishment of hyphae in the stem before autumnal defoliation. Many pycnidia which form later in the season as the area of infection proceeds from the tip of the twig towards the base are often temporarily halted in their development by the approach of winter, in which case they complete their development in early spring and their spores initiate the primary cycles of the new season.

Twigs bearing pycnidia which had not already matured and sporulated were collected in late summer from both naturally inoculated trees in the field and from artificially inoculated plants

in the greenhouse and were overwintered in screen packets on the ground. Examination of the overwintered material in the spring revealed that many of the pycnidia were exuding an abundance of spores in typical pinkish droplets. These were found to be quite viable. When water suspensions of them were placed on potato dextrose agar in petri dishes numerous colonies of the elm *Cephalosporium* developed. Spores from overwintered pycnidia on twigs collected from infected trees in early spring, likewise have proved to be viable by similar culture methods. There was no indication whatsoever of an ascomycetous stage being developed in any of the overwintered material on the ground or on the tree.

It has not only been proved that the fungus overwinters in the form of pycnidia, but also as mycelium in the previously infected twigs and branches. After the pathogene has once become established in a twig hyphal growth may continue from season to season, gradually involving more and more of the branch. Progress of infection continues from year to year in the older branches in the newly formed sapwood, the fungus growing much more rapidly in the newly formed vessels than in the older ones. From such previously infected branches hyphae grow into young suckers and other side branches each year, resulting in their death, and commonly the formation of multitudes of pycnidia in the killed bark.

When the pathogene is once established in a tree, branches are not only gradually killed by continual growth of the mycelium, but new twigs become infected each season through the wounded leaves. There appear to be many more leaves infected on a diseased tree than the number of twigs eventually killed, indicating that the pathogene may become established in the stem in only a relatively few instances each season. However, since there are so many newly infected leaves and they become more numerous year after year as a result of an increased number of dead twigs and consequent increased supply of inoculum, the number of infections which continue in the stem is still quite large. By the relatively slow but continual growth of hyphae in the tree from season to season, and especially by the multiplication of numerous new infections each additional season, more and more of the top is affected, causing the entire tree to become weakened and finally killed. Hyphae of the pathogene rarely ever reach the crown of the trunk before the entire tree is killed. Many secondary fungi

follow Cephalosporium infection, but what part they play in the final destruction of the tree has not been investigated.

Infection resulting from the elm *Cephalosporium* seems to be due to liberation of toxins or enzymes or a combination of both by the pathogene, as is indicated by lack of stored starch in the vicinity of growing hyphae and the appearance of symptoms of infection in advance of hyphal growth.

When the pathogene enters the stem some distance from the tip it is a matter of common observation that infection proceeds upwards at a much more rapid rate than downwards. When such occurs, occlusion of vessels by tyloses, gum-like substances and to some extent by the hyphae of the pathogene, appears to contribute materially to the upward progress of infection.

From the above account it is quite clear that the Cephalosporium disease cannot be classified with the so-called wilt diseases even though the malady is essentially a vascular mycosis. According to Brown (1936), "The essential feature of a wilt disease is that the parasite enters the root system and after traversing the cortex, in which it may produce relatively little disturbance, it reaches a striking development in the vascular system. The functioning of the latter is interfered with, and as a result the shoot wilts and the whole plant dies". In the case of the Cephalosporium disease, the general progress of infection is in the opposite direction to that in the wilt diseases.

CONTROL

A. PRUNING TESTS

Unlike the Dutch elm disease and the Verticillium wilt, the Cephalosporium disease of elms is more or less restricted to the twig tips and ends of branches and the symptoms of disease are exhibited clearly before the pathogene has become established in the main branches and trunk. Based on the knowledge that infection occurs in the branch tips and works relatively slowly towards the main branches and trunk and that the extent of infection is always quite clearly indicated by a marked brown discoloration in the sapwood and cambium area, an attempt was made to eradicate the pathogene from individual trees by judiciously pruning out all diseased twigs and branches. This process not only removes infected portions from the tree but also eliminates future sources of inoculum. For experimental purposes nursery

trees three to five inches in diameter at the base and 12 to 15 feet in height in a severely infested area of the elm *Cephalosporium* were carefully selected.

Preliminary tests were conducted during the summer of 1934, in which five trees were pruned and four were selected as checks. Of those pruned, two were slightly diseased, only twig tips being infected; one was moderately diseased, several smaller branches as well as a number of the twig tips being infected; and two were severely diseased, one or more of the larger branches, several of the smaller ones, and a number of the twigs being infected. As controls four trees were selected so as to compare in degrees of infection as favorably as possible with those pruned; these included two slightly, one moderately, and one severely infected tree. Each tree was given a number, notes were recorded as to amount and nature of infection, and photographs were made before and after pruning. Each branch was carefully examined and all infected branches were removed several inches, and even farther back when possible, beyond the last trace of sapwood discoloration. In the two severely diseased trees some brown discoloration had to be left in the main trunk, but all was removed from the other three. Immediately after each was pruned, all wounds were carefully painted with an antiseptic and water-proof wound dressing. All trees of this preliminary test were pruned during the first week of September, 1934. Notes were taken and photographs were made of each selected pruned and unpruned tree at frequent intervals during the following seasons of 1935 and 1936. Those which had been pruned were carefully examined and any additional infected twigs or branches were removed.

The results, as recorded in 1936, showed that the pathogene was completely eradicated in three out of five of the previously infected trees. The other two in which all of the discoloration could not be removed with the larger infected branches were still diseased when the last observation was made. Infection continued in all of the unpruned controls.

In 1935, 17 additional infected trees were pruned and 17 others comparing favorably in degrees of infection were selected as checks. The degree of infection in these trees ranged from slight to severe; in approximately one-half of them infection was slight, infection being limited to relatively few twigs; in one-quarter of them infection was moderate, infection being restricted to twigs

and small branches; and in one-quarter infection was severe, diseased areas including many twigs, smaller branches and several larger ones. Procedure was the same as that followed in the preliminary tests of 1934. In instances where not all of the discoloration could be removed in a large infected branch, a spot of red paint was placed near the cut so that any further development could be traced.

Thorough examination in 1936, one year after pruning, showed that the disease had been completely eradicated from 4 trees and materially reduced in most of the others pruned. It is reasonably certain that further pruning done in 1936 will result in complete eradication of infected areas from many more of them; previous tests have indicated that it is often necessary to go over even a slightly infected tree two successive seasons to get all of the infected twigs. The results of this experiment further indicate that the removal of infected and killed branches from diseased trees reduces the number of new infections the following year. Over three times as many infected leaves appeared on the unpruned trees in comparison with those pruned. It is believed that most if not all leaf infections occurring on the pruned trees resulted from the abundant sources of inoculum provided by near-by severely infected trees which had not been pruned.

Attempts to eradicate the disease from several large shade trees have yielded promising results. In one of these trees several of the branches in the top had been killed back 12 to 15 feet and many smaller branches and twigs distributed over the tree were infected. The tree was carefully examined and all infected branches that could be detected were removed during the summer of 1934 by a commercial tree service company under the writer's general supervision. In several instances all of the discoloration in the wood could not be removed; the remaining discoloration was in the 1932 growth ring or layer of sapwood of the larger branches. About five truck loads of infected branches were hauled away after the first pruning. In 1935 the three exhibited an abundance of new growth but there still remained a few infected branches and twigs. It was again thoroughly examined and all infected branches that could be detected were removed. This time less than half of one truck load of infected material was removed. In 1936 the tree showed still more improvement and the present indications are that the disease can eventually be eliminated. This tree was also given several applications of Bordeaux mixture and lead arsenate

each season after the pruning treatment was begun. Another large tree, though less severely infected, was apparently freed of the disease by the first pruning in 1934; no further symptoms appeared in 1935 or 1936.

The results of these tests indicate that the Cephalosporium disease of elms can be controlled by pruning if it is done before the pathogene is established in the larger branches and main trunk. When the tree is severely diseased and infection has become well established in the larger branches and trunk, it cannot be saved by pruning. Unless all areas of infection can be removed failure commonly results.

B. FUNGICIDAL AND INSECTICIDAL TESTS

Infected twigs and branches may be removed from a diseased tree and the pathogene completely eradicated, but that same tree is subject to reinfection through the commonly abundant leaf wounds when there is a source of inoculum near-by. It is obvious, therefore, that pruning alone will not satisfactorily control the Cephalosporium disease unless all sources of nearby inoculum are removed or the suscept is protected in some way against reinfection.

Since the principal infection courts are insect feeding wounds in leaves, the control of leaf-eating insects by spraying the trees with an insecticide or covering of the open leaf wound with a protective fungicide should theoretically aid in controlling the disease. With this possibility in mind certain tests have been conducted with both fungicides and insecticides.

Laboratory fungicidal tests

Following the method of testing protective fungicides in the laboratory as outlined by McCallan (1930), with slight modification, the toxicity of copper and sulphur to germinating spores of the elm *Cephalosporium* was tested. The method consisted in spraying the fungicide to be tested uniformly over a number of chemically clean and dry microslides. After they were thoroughly dry four were placed in each of a number of moist chambers of uniform size on racks made of glass tubing bent to form a U. A spore suspension was prepared from a culture on the sugar-free synthetic nutrient agar medium two to four weeks old using sterile water. Four drops of spore suspension from a sterile pipette were placed on each slide equidistant apart, thus making a total

of 16 drops in a single moist chamber. Only one fungicide was used in each chamber during any one test. After spores were well germinated in the checks, or in the drops of water on slides without the fungicide, counts of the relative number of germinating spores in each were made; this was usually 48 hours after the drops of spore suspension were placed on the slides. Since ungerminated spores are so minute, only the germinated ones were counted in one low-power field near the center of each drop. It was therefore impossible to compute the percentage of germination, but, nevertheless, the relative numbers of germinated spores in each drop were comparable and provided an adequate basis of comparison. These tests were conducted as three separate experiments.

Experiment I.

The purpose of the first experiment was to determine whether or not a copper fungicide, Bordeaux mixture, is toxic to the germinating spores of the elm *Cephalosporium*. Both the ordinary Bordeaux mixture (4—4—50) and a commercial preparation known as Oxo-Bordeaux manufactured by the Ansbacher-Siegle Corp. in Brooklyn, New York, were tested. The Oxo-Bordeaux was used at the rate of eight pounds to 100 gallons of water, which is equal to the strength of 4—4—50 Bordeaux mixture. The relative amounts of spore germination in the presence of the fungicides were tested against the relative amounts germinating on clean slides in drops of sterile water. Summarizing the results of six series in which one field in each of 240 drops for each fungicide and for the control was examined, the average number of germinated spores per field in the 4—4—50 Bordeaux, Oxo-Bordeaux, and water was 00.55, 00.32 and 28.77, respectively. These data clearly demonstrate that both fungicides are toxic to the germinating spores of the pathogene.

Experiment II.

Since it was found that Bordeaux mixture at the strength of 4—4—50 is toxic to germinating spores of this pathogene, it seemed desirable to determine what strength of the fungicide would be most effective. Similar tests were conducted in which four different strengths of Bordeaux mixture were used, namely, 1—1—50, 2—2—50, 4—4—50 and 8—8—50. The results of this test, involving one series of 16 drops for each strength of fungicide and for the control, showed an average number of 64.06 germinated

spores per field in water as compared with 4.06 in 1—1—50, 1.60 in 2—2—50, 1.19 in 4—4—50 and 0.13 in 8—8—50 Bordeaux mixture. It is, therefore, clearly demonstrated that spore germination is materially reduced in even the weakest mixture, but is reduced to a greater degree in the more concentrated solutions.

Experiment III.

In this experiment the toxicity of various strengths of Linco colloidal sulphur to germinating spores of the pathogene was investigated. Six different strengths of the fungicide were used, namely, 1, 2, 4, 8, 12 and 16 pounds per 100 gallons of water. This experiment was conducted in the same manner as that of the preceding two. The results indicated that even at the higher concentrations a large number of the spores germinated in the presence of the fungicide, there being an average of 26.63 germinated spores per field in water as compared with 12.53 in the preparation containing 16 pounds of sulphur per 100 gallons of water. A strength of eight pounds of this fungicide per 100 gallons of water is considered a rather highly concentrated solution to be used in spraying plants; yet 16 pounds per 100 gallons of water only reduced spore germination to about one-half of that occuring in water alone.

Fungicidal tests on seedlings in the greenhouse

The effectiveness of Bordeaux mixture and Linco colloidal sulphur in protecting wounded leaves against invasion by the elm *Cephalosporium* was tested in the greenhouse on artificially inoculated seedlings of American elm. The leaves of 10 plants were atomized with Bordeaux mixture (4—4—50), the spray material was allowed to dry on the leaves, and then 5 leaves per plant were wounded by severing a main lateral vein in each with a sterile scalpel; 10 other plants were wounded first and then sprayed with the fungicide. Twenty other plants were similarly wounded and sprayed with Linco colloidal sulphur (8 pounds per 100 gals. water) and 10 plants to be used as checks against those sprayed were similarly wounded but were not sprayed. All were inoculated with spore suspension from the same preparation. They were then placed in a moist chamber for 72 hours. Results indicated an insignificant reduction of infection when sprayed with either fungicide, except that there was a 60 per cent reduction in the case of colloidal sulphur applied after the leaves were wounded.

FUNGICIDAL AND INSECTICIDAL TESTS ON NURSERY TREES

The effectiveness of spraying a combination fungicide and insecticide on the foliage of nursery trees as a possible means of controlling the Cephalosporium disease was investigated. As a preliminary test three blocks of American elms 12 to 15 feet in height, containing a number of infected specimens, were selected. In cooperation with a commercial arborist half of the trees of each block were sprayed with Oxo-Bordeaux (8 pounds per 100 gallons of water) and arsenate of lead (4 pounds per 100 gallons of water) with a power sprayer. One application was made on May 15 and another on June 1 during the season of 1935. Observations taken at frequent intervals throughout the season clearly showed that injuries caused by canker worms, spiny elm caterpillars, and other leaf-eating insects were materially reduced by spraying. Gross examination also indicated that the numbers of leaf infections were fewer on the trees which were sprayed.

In 1936, two rows of American elms of the same size as those used in the preliminary test were selected for more intensive tests in which the same Bordeaux mixture and arsenate of lead combination was used. The numbers of infected and uninfected trees were essentially the same in each row; a complete record of each tree had been made during the two preceding seasons. One row was sprayed and the other was retained as a check; four applications were made at two-week intervals beginning April 15 and ending June 1. In addition to Bordeaux and lead the second application contained Blackleaf 40. The twigs, branches and trunks, as well as the leaves, were thoroughly coated with the spray material. Notes were taken at frequent intervals throughout the season regarding insect injury and Cephalosporium infection of each individual tree. All sprayed trees exhibited very little leaf injury while those which were not sprayed were severely injured by leaf-eating insects. The leaves of the unsprayed trees were severely wounded by canker worms and spiny elm caterpillars. During the last week in July each tree was thoroughly examined and the numbers of primarily infected leaves were counted, a summary of which showed an average of 10.2 infected leaves per tree on those sprayed as compared with 11.7 on those not sprayed; the sprayed row involved 48 trees while the unsprayed row involved 37. The slight reduction in number of leaf lesions did not appear to be in proportion to the large reduction of insect injury. Whether the fungicide offered any pro-

tection against the pathogene entering the leaf wounds was not indicated.

Similar tests have been conducted by Carter (1935) in which the effectiveness of Bordeaux mixture, sulphur sprays and sulphur dusts was tested in controlling elm "wilt" diseases in Illinois nurseries. His tests, extending over a period of 4 years, indicated that the applications of sulphur dusts reduced the number of new infections, although in most instances the reduction was not great. Bordeaux mixture was less effective than the dusts and the sulphur sprays were still less effective. Whether the Cephalosporium disease was included among the elm "wilt" diseases referred to by Carter was not stated in his paper.

Further investigations on the control of the Cephalosporium disease by means of fungicides and insecticides should be continued. Other fungicides should be tested in the laboratory and greenhouse under controlled conditions as well as in the nursery where conditions cannot be so well controlled. There are several difficulties in attempting to control the Cephalosporium disease by applying spray materials to the leaves; these have been encountered in the course of the experiments discussed above. When a stomach poison, such as arsenate of lead, is employed as an insecticide the insect must eat a sizable hole in the leaf before enough poison is consumed to be fatal; a suitable infection court for the elm *Cephalosporium* is therefore inflicted. Furthermore, a leaf may be thoroughly coated with an effective protective fungicide but any insect wound inflicted after the fungicide has been applied immediately provides an unprotected surface through which the pathogene may enter. These are a few of the difficulties which must be overcome if fungicides and insecticides are to be entirely effective in controlling the Cephalosporium disease.

C. CONTROL RECOMMENDATIONS

Based on the foregoing facts brought out in life history studies and control tests the following recommendations, some of which have been previously outlined (Creager, 1936), are given for combating the Cephalosporium disease.

1. All infected trees should be excluded from ornamental plantings and nurseries. The disease is definitely contagious. The introduction of diseased stock seems to be the common origin of epidemics. If at all possible trees which are to be moved into

ornamental plantings from another locality should be carefully examined for the symptoms of this disease during the preceding growing season.

2. Slightly to moderately diseased trees may be successfully freed of infection by carefully pruning out all infected branches. This method seems to be the only means of eliminating the trouble from individual trees. The characteristic symptoms, externally as well as internally, are the guides to follow in pruning. In all cases the branch should be removed quite a distance back of the discoloration in the sapwood in order to be sure that all of the fungus has been removed. It is usually necessary to repeat the process several successive seasons before all of the infected branches and twigs are finally eradicated, since slightly infected twigs are often difficult or impossible to detect at the outset. As is to be expected in such trees as are very extensively diseased, all infected trees cannot be saved by this method; the slighter the infection the greater the chance one has for success in pruning. All infected branches should be burned.

3. All badly infected trees in ornamental plantings and nurseries, which are too badly infected to be saved by pruning or their value does not warrant the time and expense of such an operation, should be eradicated. Such trees are constant sources of danger to more valuable trees on the lawn or in the nursery. The infected twigs and branches, at least, should be immediately burned, as it is in the twigs and smaller branches that pycnidia form.

4. Since wounds in leaves are the principal infection courts, trees should be kept as free as possible from insect injury, especially those trees that are or have been infected and those that are in the vicinity of infected neighbors. By the application of a combination of insecticidal and fungicidal spray the number of leaf infections may be reduced.

SUMMARY AND CONCLUSIONS

1. Almost coincident with the discovery in 1930 of the Dutch elm disease in the United States attention was called to a similar native disease of elms that was found to be the cause of hitherto baffling losses in nurseries, plantations and natural stands — a disease known as "elm die-back" or "Cephalosporium wilt" and here tentatively designated "Cephalosporium disease". This paper presents the results of a comprehensive study of this disease.

2. The Cephalosporium disease occurs almost throughout the natural range of *Ulmus americana*. It is not known to occur outside of the United States and Canada. The American elm is by far its most important suscept though in several instances the disease has been reported on *U. fulva* and *U. pumila*. Under laboratory conditions *U. japonica* was also found to be susceptible.

3. In addition to those symptoms and signs already more or less generally recognized, this paper adds the following: (a) a comprehensive account of symptoms as they occur in the field; (b) the discovery of primary leaf lesions; (c) the discovery of the production of pycnidia on a large scale.

4. Etiological studies may be summarized as follows:

(a) Extensive inoculation tests prove beyond doubt that the disease is caused by a fungus. This fungus produces two types of fructifications — one of a hyphomycetous type, the other a sphaerioidaceous type. A detailed study of both shows, for the first time, that the spores are essentially "endoconidia". The taxonomic position of the pathogene is problematic; the most that can be said is that on some media it appears to be a good *Cephalosporium*, on others it is more closely related to the genus *Cadophora*, and on still others it develops pycnidia which relate it with members of the Sphaerioidaceae.

(b) Cultures from various sources confirm the view that several strains are involved, differing in cultural features and pathogenicity.

(c) Some new data relative to the characteristic crystals that are produced in some cultures are described.

(d) Heretofore, difficulty has been experienced in obtaining Cephalosporium fructifications. These fructifications were obtained in great abundance on a synthetic nutrient agar medium lacking sugar. This facilitates identification and enables one to obtain a large and uniform supply of spores for inoculation studies.

(e) Detailed culture studies have been made with regard to the effects of temperature, sugar concentration and various types of natural and artificial media.

(f) A comprehensive study has been made of the phenomena of infection. Most important among the results it has been shown for the first time (1) that entrance is commonly effected

through leaf wounds, such for example as those inflicted by the canker worms and other leaf-eating insects — a feature of primary importance in devising means of control; (2) that pycnidia occur abundantly on overwintered twigs killed by the fungus and that their spores are of very great significance in the spread of the disease — also a fact of primary importance with respect to control; (3) that wind, rain, and insects appear to be important factors in spore dissemination; (4) that the course of infection follows the vascular system of leaf veins into the vascular system of the stem; and (5) that the mycelium invades not only the vascular system but subsequently the wood parenchyma, rays, pith, cambium, phloëm, and all parenchymatous cells of the bark.

5. Studies on the control of the Cephalosporium disease have demonstrated the following:

   (a) The pathogene may be eradicated from slightly to moderately infected trees by judicious pruning.

   (b) Bordeaux mixture is toxic to germinating spores of the elm *Cephalosporium.*

   (c) Spraying infected and near-by uninfected trees with an insecticide-fungicide reduces the number of leaf infections.

6. Recommendations for combating the Cephalosporium disease, based on life history studies and control tests, are listed.

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LITERATURE CITED

1. Andrus, C. F. & L. L. Harter. Morphology of reproduction in Ceratostomella fimbriata. (Jour. Agr. Res. **46**:1059–1078. 1933.)
2. Anonymous. Report of the director for the year ending October 31, 1930. Elm disease under investigation. (Connecticut Agr. Exp. Sta. Bull. **322**:119. 1931.)
3. ————Report of the director for the year ending October 31, 1931. Elm fungus destroying trees. (Connecticut Agr. Exp. Sta. Bull. **337**: 462. 1932.)
4. ————Cephalosporium wilt and die-back of elm. (New Jersey Agr. Exp. Sta. Nursery Disease Notes **7**:23– 28. 1934.)
5. ————(Plant Disease Reporter, Suppl. **86**: 70. 1935.)
6. Beattie, R. Kent & G. F. Gravatt. (Plant Disease Reporter, Suppl. **85**: 61. 1933.)
7. Boyd, O. C. Some reports on plant diseases in Massachusetts. (Plant Disease Reporter **18**: 126. 1934.)
8. Brown, W. The physiology of host-parasite relations. (Bot. Rev. **2**: 236–282. 1936.)
9. Buisman, Christine. Verslag over de onderzoekingen betreffende de iepenziekte, verricht in het Phytopathologisch Laboratorium "Willie Commelin Scholten" te Baarn, gedurende 1934. (Tijdschr. Plantenziek. **41**:104–120. 1935.)
10. Carter, J. C. A report on tests of fungicides for the control of elm diseases in nurseries. (Illinois Nat. Hist. Surv. Biol. Notes **4**:1–7. 1935.)
11. Corda, A. C. I. Icon. Fungorum **3**:11 (1839).
12. Creager, D. B. New facts concerning Cephalosporium wilt of elms. (Jour. Arnold Arb. **16**:453– 454. 1935.)
13. ————Cephalosporium wilt of elm. (Proc. Twelfth Nat. Shade Tree Conf. pp. 140–144. 1936.)
14. Davidson, R. W. Fungi causing stain in logs and lumber in the southern States including five new species. (Jour. Agr. Res. **50**: 789–807. 1935.)
15. Davis, W. H. A disease survey of trees and shrubs in western Massachusetts. (Plant Disease Reporter **19**: 260–264. 1935.)
16. Dodge, B. O. The non-sexual and the sexual functions of microconidia of Neurospora. (Bull. Torrey Bot. Club **59**: 347–360. 1932.)
17. Gerry, E. Tyloses: their occurrence and practical significance in some American woods. (Jour. Agr. Res. **1**:445–469. 1914.)
18. Gilbert, W. W. The root-rot of tobacco caused by Thielavia basicola. (U.S.D.A. Bur. Plant Ind. Bull. **158**:1-55. 1909.)
19. Goss, R. W. & P. R. Frink. Cephalosporium wilt and die-back of the white elm. (Univ. Nebraska Agr. Exp. Sta. Res. Bull. **70**: 1–24. 1934.)
20. Gwynne-Vaughan, H. C. I. & G. E. Broadhead. Contributions to the study of Ceratostomella fimbriata. (Ann. Bot. **50**: 747–758. 1936.)

21. Harris, Hubert A. Initial studies of American elm diseases in Illinois. (Illinois Nat. Hist. Surv. Bull. **20**:1–70. 1932.)
22. Knudson, Lewis. Tannic acid fermentation I. (Jour. Biol. Chem. **14**:159–202. 1913.)
23. Lagerberg, T., G. Lundberg & E. Melin. Biological and practical researches into blueing in pine and spruce. (Svenska Skogsvårdsför. Tidskr. **25**:145–739. 1927.)
24. Liming, O. N. Elm diseases in America. (Phytopath. **23**: 21. 1933.)
25. ————The Dutch elm disease eradication program in the United States. (Proc. Tenth Nat. Shade Tree Conf. pp. 67–72. 1934.)
26. Mason, E. W. Annotated account of fungi received at the Imperial Mycological Institute. List II, fascicle 2. pp. 1–67. (Imperial Mycological Institute, Kew, Surrey. 1933.)
27. May, Curtis. A new elm disease. (Science, n. s. **74**: 437. 1931.)
28. ————Notes on the work of the Dutch Elm Disease Laboratory. (Proc. Tenth Nat. Shade Tree Conf. pp. 73–75. 1934.)
29. McCallan, S. E. A. & H. H. Whetzel. Studies on fungicides. (Cornell Univ. Agr. Exp. Sta. Memoir **128**: 3–79. 1930.)
30. Melin, E. & J. A. Nannfeldt. Researches into the blueing of ground woodpulp. (Svenska Skogsvårdsför. Tidskr. **32**:397–616. 1934.)
31. Pinkerton, M. E. A comparative study of conidial formation in Cephalosporium and some related Hyphomycetes. (Ann. Missouri Bot. Garden **23**:1–68. 1936.)
32. Rudolph, B. A. Verticillium hadromycosis. (Hilgardia, **5**:197–354. 1931.)
33. Schwarz, M. B. Das Zweigsterben der Ulmen, Trauerweiden und Pfirsichbäume, ein vergleichend-pathologische Studie. Utrecht, A. Oosthoek, pp. 7–32. 1922. (Transl. by Lillian D. Kelsey in Bartlett Research Laboratories Bull. **1**:5–25. 1928.)
34. Tehon, L. R. Elm diseases in Illinois. (Proc. Tenth Nat. Shade Tree Conf. pp. 105–111. 1934.)
35. ————& H. L. Jacobs. A Verticillium root disease of American elm. (Davey Tree Expert Co. Bull. **6**: 3–32. 1936.)
36. Verrall, A. F. Die-back of elm in Minnesota. (Phytopath. **20**:1004–1005. 1930.)
37. ————(Plant Disease Reporter, Suppl. **90**:100. 1935.)
38. ————& Curtis May. A new species of Dothiorella causing die-back of elm. (Mycologia **29**: 321-324. 1937.)
39. Wilson, J. K. Calcium hypochlorite as a seed sterilizer. (Am. Jour. Bot. **2**:420–427. 1915.)

EXPLANATION OF PLATES

PLATE I

A young tree of *Ulmus americana* affected with the Cephalosporium disease, exhibiting typical symptoms of die-back and blight of the twigs and smaller branches.

PLATE II

American elms affected with the Cephalosporium disease.

Figure 1. A young tree with severely infected top, exhibiting a condition commonly known as "stag-head".
Figure 2. A young tree showing symptoms of severe blight.
Figure 3. A large tree exhibiting symptoms of die-back and abundant sucker formation along the larger branches.
Figure 4. A large tree with its infected top markedly deformed, showing many killed branches and groups of sucker-growth.

PLATE III

Leaves and twig tips of *Ulmus americana* exhibiting symptoms of Cephalosporium infection.

Figure 1. Primary leaf lesions originating at borders of wounds inflicted by larvae of the fall canker worm, *Alsophila pometaria* Harr.
Figure 2. A more advanced stage of primary leaf lesion with a brown necrotic area near the infection court separated from the lower green area of the leaf by a marked yellowish zone; at such a late stage a brown discoloration commonly extends through the vascular strand of the mid-rib, petiole, and leaf traces into the stem.
Figure 3. Symptoms of defoliation and "die-back" of stem tip following current season's infection through wounded leaves.
Figure 4. Early spring symptoms of blight exhibited by young lateral shoots immediately below a killed stem tip which became infected through leaves during the preceding season. Collected and photographed June 4, 1936.

PLATE IV

Symptoms exhibited in stems of *Ulmus americana* infected by the elm *Cephalosporium*.

Figure 1. Pieces of infected stems showing twig scars, created by abscission of lateral branches along the infected sides, and elongated bark cankers in various degrees of formation.
Figure 2. Stems with portions of the bark removed showing the characteristic diffuse brown discoloration in the cambium and sapwood area.

Figure 3. A stem with the bark removed, showing the less typical narrower discolored streaks in the infected sapwood.
Figures 4 and 5. Stems cut diagonally showing crescent shaped areas of discoloration involving sapwood, cambium and bark.
Figure 6. Stem cut diagonally showing discontinuous flecks in the outer layers of sapwood (bark not affected).

PLATE V

Discolored leaf traces caused by ingress of the elm *Cephalosporium* from infected leaves.

Figure 1. Young twig with bark removed showing brown discoloration leading downwards from a lateral leaf trace. × 2.
Figure 2. Young twig with bark removed and split longitudinally through an infected central leaf trace showing discolored area leading from leaf scar into the primary xylem. × 3.
Figure 3. Free-hand transverse unstained section showing a discolored lateral leaf trace and a portion of an uninfected central trace at the left. × 50.
Figure 4. Transverse section of a two-year-old twig which became infected during the preceding season, showing fan-like extensions of the discoloration from the leaf traces through the current season's sapwood to cambium and bark. × 50.

PLATE VI

Symptoms and signs exhibited in stems of *Ulmus americana* infected by the elm *Cephalosporium*.

Figure 1. An uninfected stem. × 50.
Figure 2. An infected stem showing discolored and occluded xylem vessels in transverse section. × 50.
Figure 3. Hyphae of the elm *Cephalosporium* in vessel lumina. × 250.
Figure 4. Xylem vessels of an infected stem partially occluded by tyloses. × 225.
Figure 5. Xylem vessels of an infected stem occluded by a brownish gum-like substance. × 250.
Figure 6. Transverse section of an infected stem with discoloration extending from sapwood into cambium, phloem, and parenchymatous tissues of the bark. × 50.

PLATE VII

Yellow crystals formed in cultures of the elm *Cephalosporium*.

Figure 1. Under-surface view of a petri dish culture on malt extract agar medium showing an abundance of crystals beneath the mycelial mat. × 1.

Figure 2. Portion of the same as that shown in Figure 1, showing the characteristically branched groups of crystals more highly magnified. × 5.
Figure 3. Crystals on the upper surface of the same mycelial mat showing their needle-like character. × 5.
Figure 4. Crystals derived from an alcoholic extract by evaporating the solvent. × 14.

PLATE VIII

Spore-head stage of the elm *Cephalosporium*.

Figure 1. Mucilaginous spore-heads borne at the tips of phialides as they occur on aërial hyphae in cultures on a sugar-free synthetic agar medium one week old.
Figure 2. Phialides borne on geniculately branched phialophores which were developed on aërial hyphae, showing origin and development of conidia.
Figure 3. Phialides borne on short phialophores, which developed from hyphae and stromatic groups of cells on the agar surface.
Figure 4. Conidia.
Figure 5. Germinating conidia.

PLATE IX

Pycnidia of the elm *Cephalosporium*, showing their place of origin and various stages in their development in the bark of *Ulmus americana* as seen in transverse sections of the young stem.

Figure 1. Early stage of primordium originating beneath the cork layer in the phellogen tissue.
Figure 2. Somewhat later stage showing the pseudo-parenchymatous nature of its tissue.
Figure 3. Early stage of cavity formation, the cavity being filled with spores from almost the very beginning.
Figure 4. A later stage, showing large cavity filled with spores and an erumpent ostiolar beak developing above.
Figure 5. A nearly mature pycnidium showing its rather thick wall and recently formed ostiole.
Figure 6. An older pycnidium in which the wall has become thin and spore formation has declined.

PLATE X

Seedlings of *Ulmus americana* artificially inoculated with the elm *Cephalosporium* through basal stem wounds.

Figure 1. A one-year-old seedling exhibiting symptoms of suckering above and below the infection court. Inoculated March 12, 1935 and photographed April 7, 1935.

Figure 2. A one-year-old seedling exhibiting symptoms of blight and yellowing of leaves. Inoculated March 12, 1935 and photographed April 7, 1935.
Figure 3. Inoculated plant at the left showing symptoms of severe blight—leaves curled and brown; inoculated March 12, 1935 and photographed May 20, 1935.
Uninoculated check at the right.

PLATE XI

Two-year-old seedlings of *Ulmus americana* inoculated with the elm *Cephalosporium* through stem tip wounds.

Figure 1. Early symptoms of infection, showing blighted leaf immediately above infection court. Inoculated April 12, 1935; photographed May 18, 1935.
Figure 2. Inoculated plant with one branch showing symptoms of partial defoliation and the other showing no symptoms at all. Inoculated April 12, 1935; photographed September 30, 1935.
Figure 3. Plant exhibiting symptoms of die-back in both inoculated branches but restricted to that portion of stem beyond the infection court. Inoculated April 12, 1935; photographed September 30, 1935.
Figure 4. A plant exhibiting a more advanced stage of die-back. Inoculated April 12, 1935; photographed September 30, 1935.
Figure 5. Pycnidia of the pathogene developed in an artificially inoculated and subsequently killed twig such as those illustrated in Figures 3 and 4. Stem inoculated April 12, 1935; photographed October 9, 1935.

PLATE XII

Symptoms exhibited by leaves of *Ulmus americana* following artificial inoculation with the elm *Cephalosporium*.

Figure 1. Leaf of a one-year-old seedling artificially inoculated through a wound in which a main lateral vein was severed, showing a marked yellowish lesion between the infection court and midrib. Inoculated March 16, 1935 and photographed April 26, 1935.
Figure 2. Advanced stages of leaf lesions in vicinity of wounds showing necrotic brown areas bordered by marked yellowish zones. Inoculated April 13, 1935 and photographed April 26, 1935.
Figure 3. A leaf artificially inoculated through a wound in which only the smaller veins were severed, showing yellow mottled infected area.
Figure 4. Early stage of infection, showing yellowish area and discolored veins in the vicinity of the infection court. × 6.5.
Figure 5. Later stage of infection, showing mottling of surrounding mesophyll and discoloration of veins and midrib. × 6.5.

PLATE XIII

Seedlings of *Ulmus americana* artificially inoculated through leaf wounds with the elm *Cephalosporium*.

Figure 1. Check plant, wounded but not inoculated.
Figure 2. An infected plant showing leaf lesions in different stages of development — all leaves were inoculated at the same time.
Figure 3. A portion of a stem with a shriveled axillary bud and a brownish necrotic area in the vicinity of the leaf scar following abscission of an inoculated leaf. × 5.
Figure 4. A plant exhibiting symptoms of die-back and darkening of the affected tip.
Figure 5. An infected stem bearing erumpent pycnidia of the pathogene. × 10.
Figure 6. Mycelium of the elm *Cephalosporium* growing from blocks of infected tissue taken from an artificially inoculated leaf such as illustrated in Figure 2.
Figure 7. Mycelium of the pathogene growing from a stem which became infected through wound-inoculated leaves such as that illustrated in Figure 4.

PLATE XIV

Two-year-old seedlings of *Ulmus americana* inoculated with the elm *Cephalosporium* through leaf wounds (5 leaves on each of the two main branches were inoculated).

Figure 1. Both inoculated branches showing symptoms of marked die-back. Inoculated April 16, 1935; photographed September 30, 1935.
Figure 2. All ten inoculated leaves developed marked lesions but only a few dropped and no die-back of stems resulted. Inoculated April 16, 1935; photographed September 30, 1935.
Figure 3. Inoculated leaves became infected and dropped but no die-back of stems occurred (inoculated at the same time with the same spore suspension and grown under the same conditions as the plant shown in Figure 2).
Figure 4. Both inoculated branches defoliated and killed from tips to nearly the main stem (inoculated with same spore suspension at same time and grown under the same conditions as those plants illustrated in Figures 2 and 3).

PLATE XV

Susceptibility of *Ulmus pumila* L. and *Ulmus japonica* Sarg. to the Cephalosporium disease — symptoms exhibited by seedlings inoculated through leaf wounds.

Figure 1. Infected leaves of *Ulmus pumila* L. inoculated with the elm *Cephalosporium* through wounds, showing yellowish to brown necrotic lesions and marked discoloration of the veins and midribs originating at the infection courts.

Figure 2. An infected seedling of *Ulmus japonica* Sarg. which was inoculated through leaf wounds with the elm *Cephalosporium*, showing brown and curled leaves along one side of the stem. Inoculated March 14, 1935; photographed May 18, 1935.

Figure 3. The same plant (*U. japonica*) as illustrated in Figure 2, exhibiting typical symptoms of die-back (tip of stem was previously removed for culturing). Photographed September 30, 1935.

PLATE XVI

Pycnidia of the elm *Cephalosporium* and infection courts in nature.

Figure 1. Groups of erumpent pycnidia of the elm *Cephalosporium* in the bark of an infected stem of *Ulmus americana*. × 5.

Figure 2. Pycnidia of the elm *Cephalosporium* as they appear on a stem with cork layer removed. × 100.

Figure 3. Mucilaginous droplet of conidia exuded from a pycnidium of the elm *Cephalosporium* projecting through the bark of a naturally infected stem as seen by transmitted light. × 350.

Figure 4. Insect-inflicted leaf wounds which have served as infection courts for the elm *Cephalosporium;* three leaves bear typical primary lesions.

Figure 5. Transverse section of a leaf midrib showing sections of hyphae of the elm *Cephalosporium* in vessel lumina. × 460.

CEPHALOSPORIUM DISEASE OF ELMS

FULL-TONE — MERIDEN

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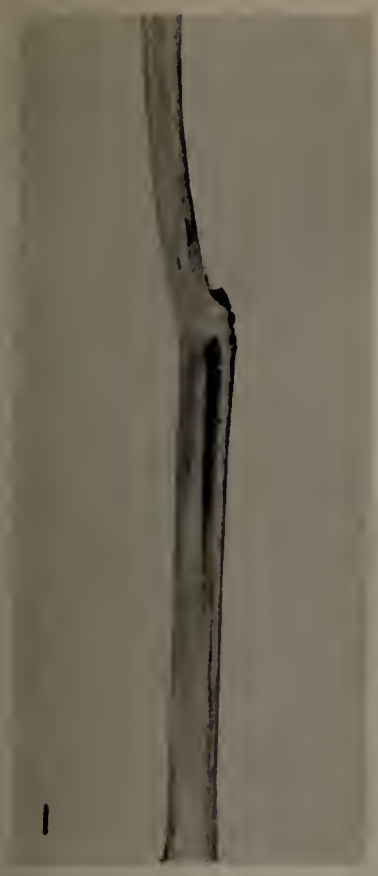
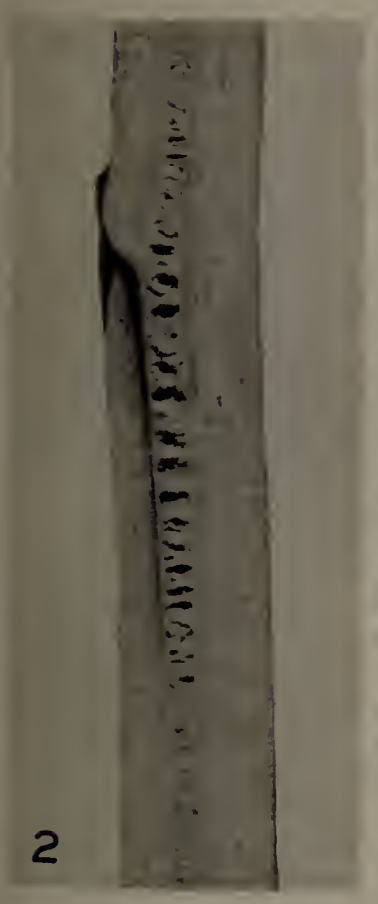
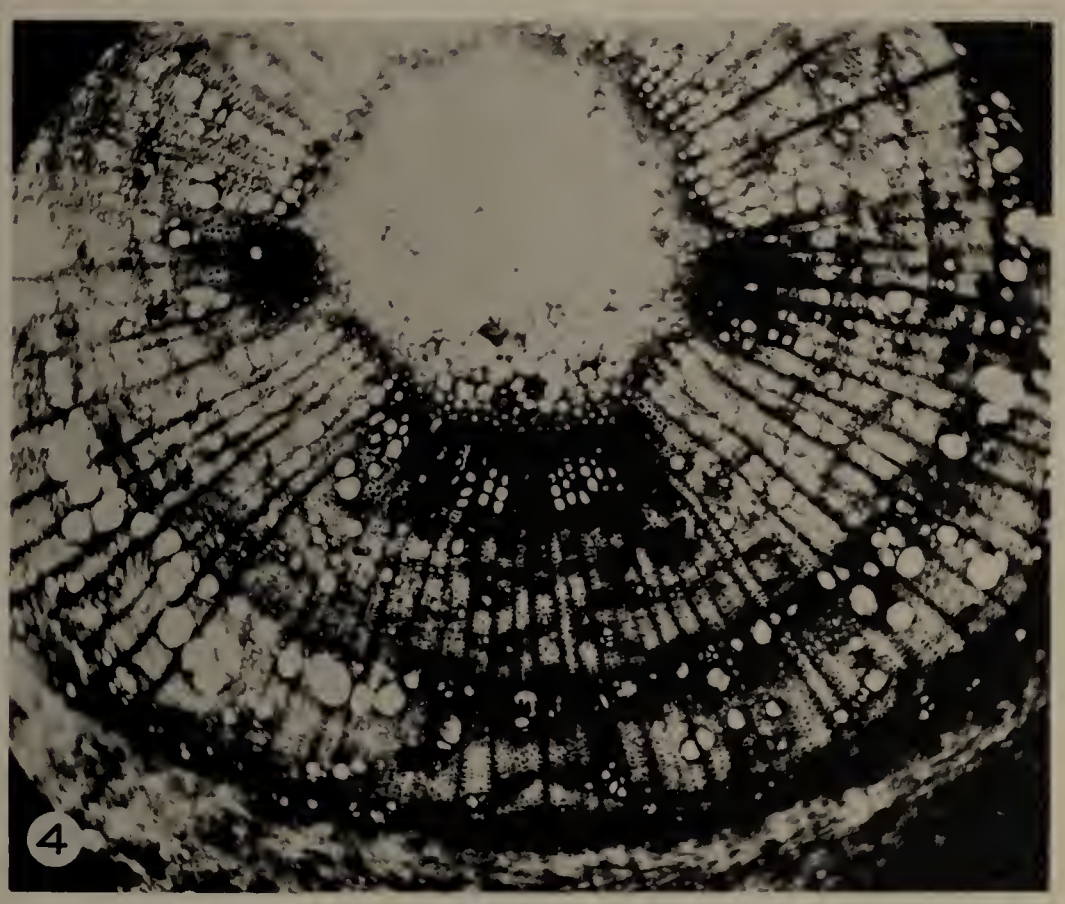
CEPHALOSPORIUM DISEASE OF ELMS

FULL-TONE — MERIDEN







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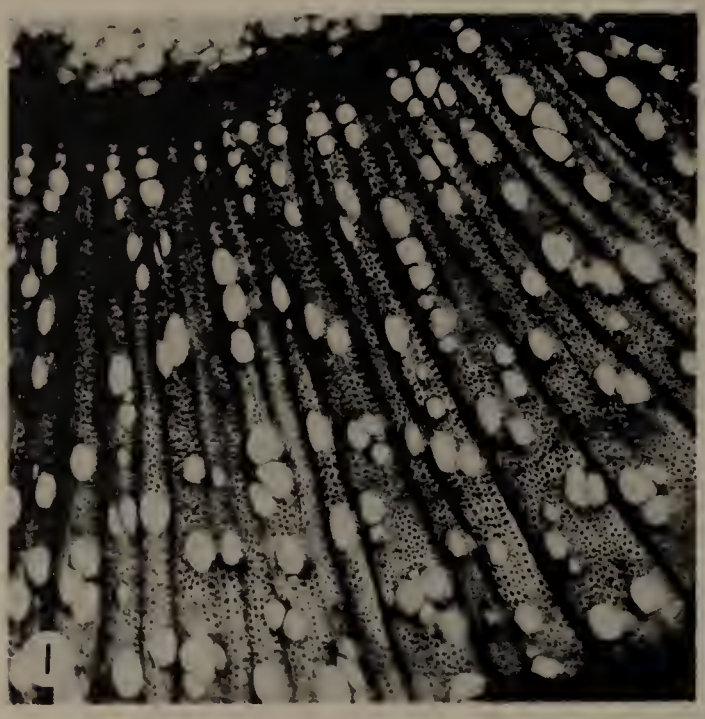
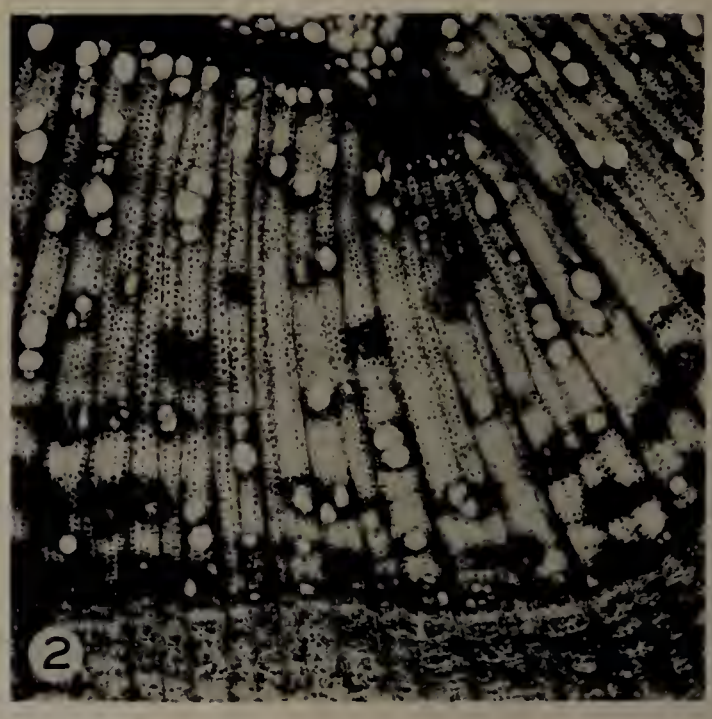
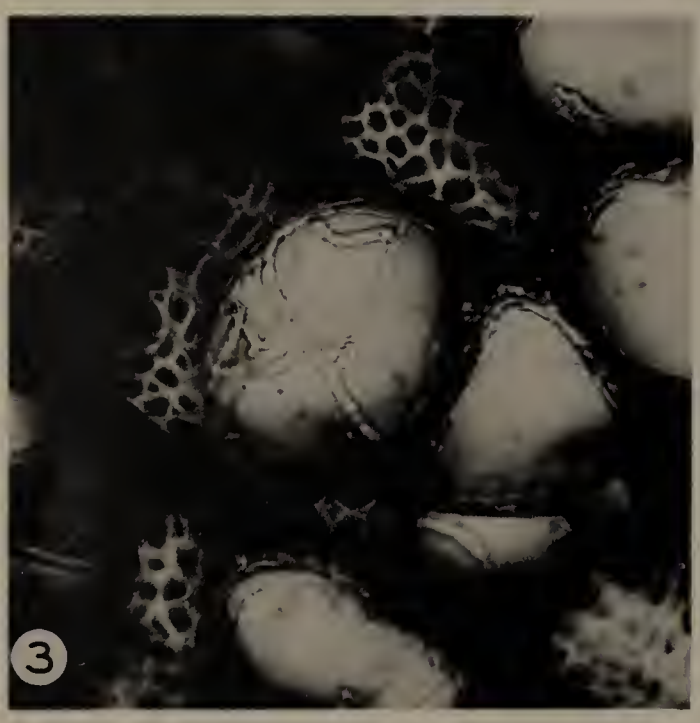
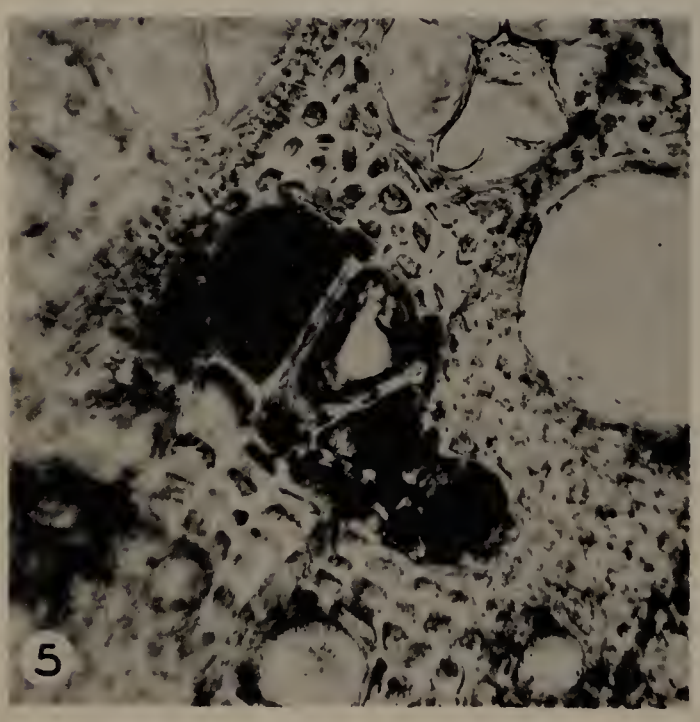
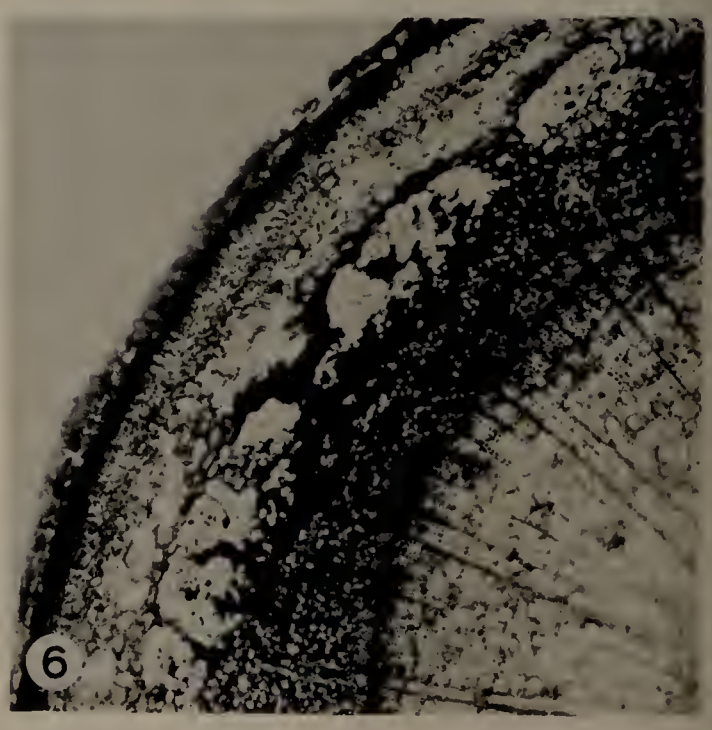
FULL-TONE — MERIDEN











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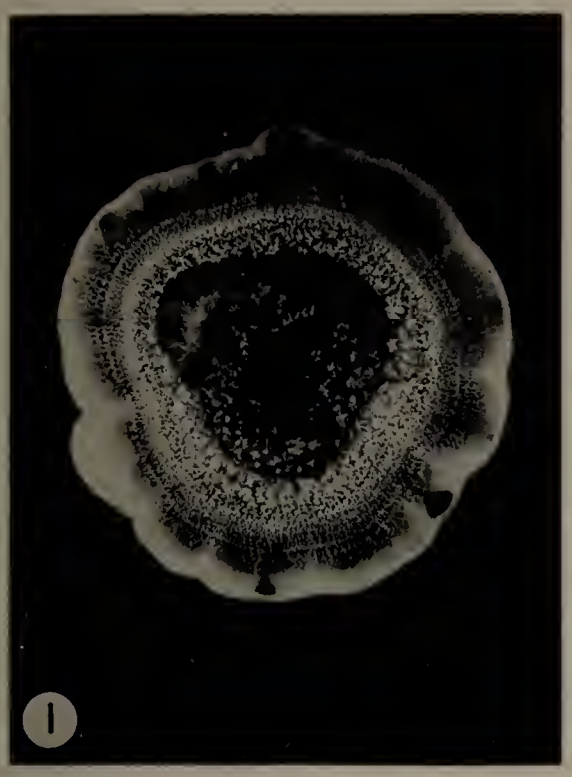

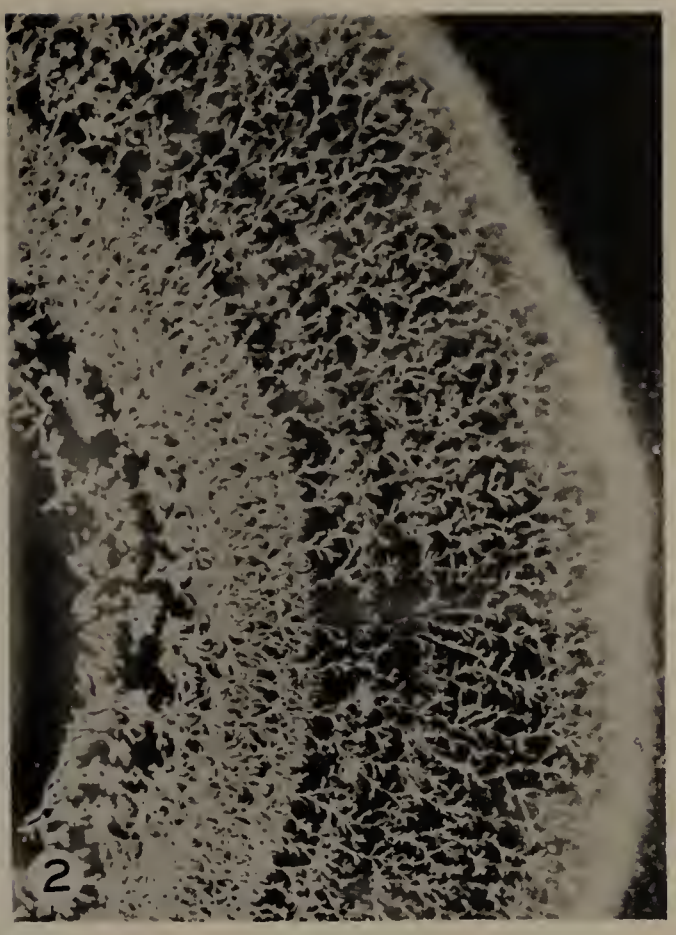

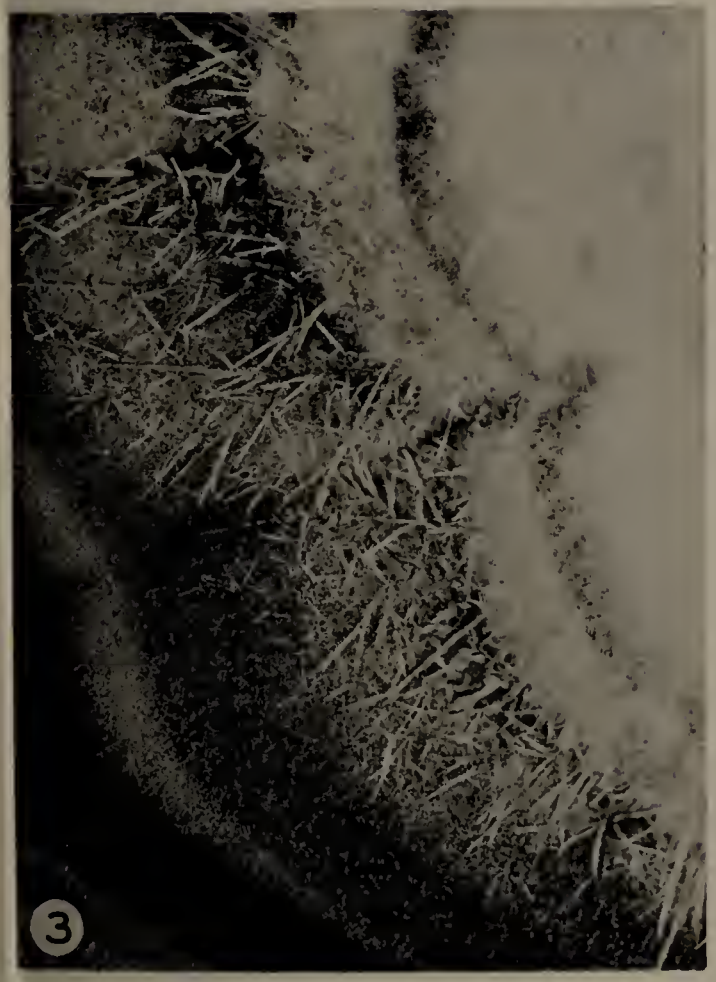

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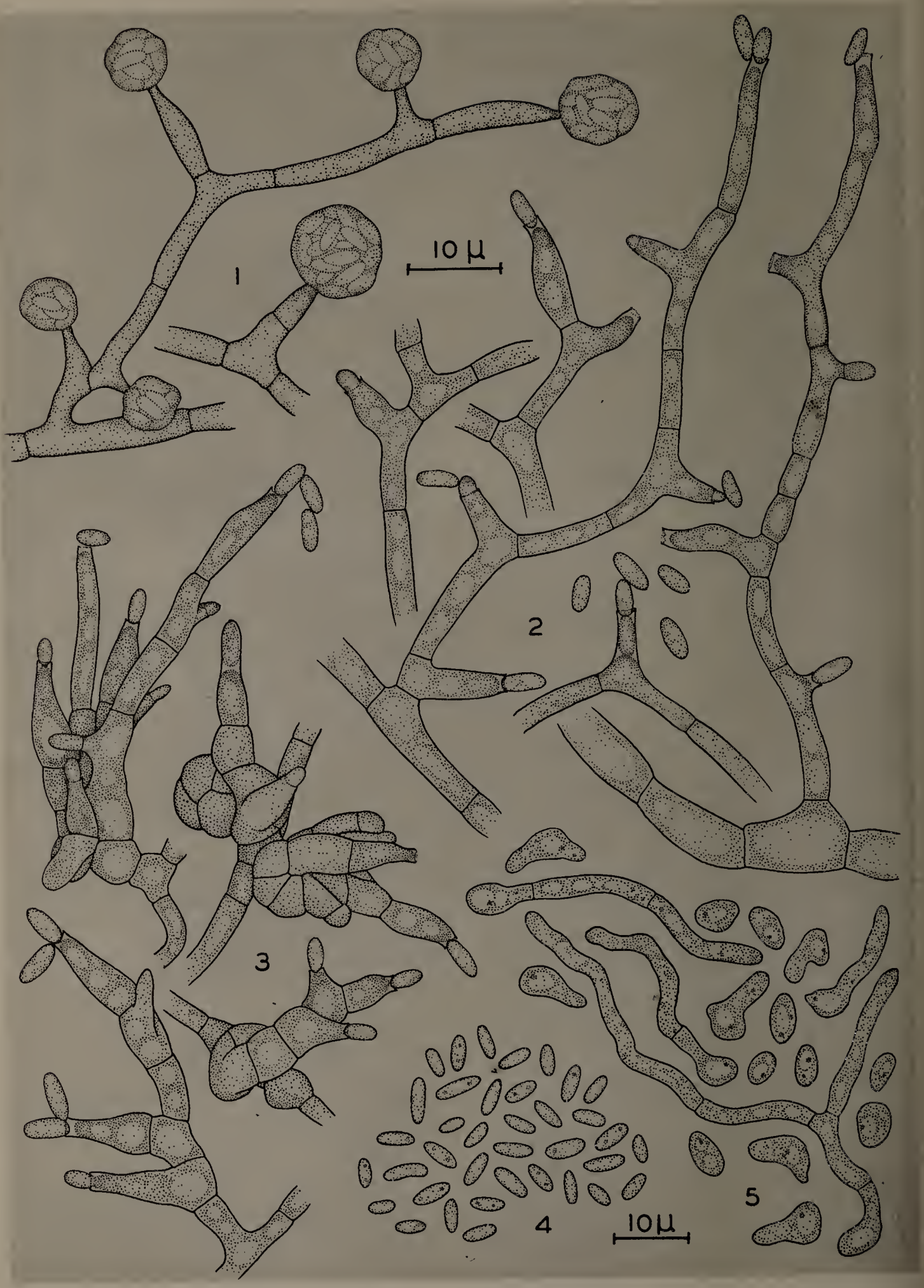

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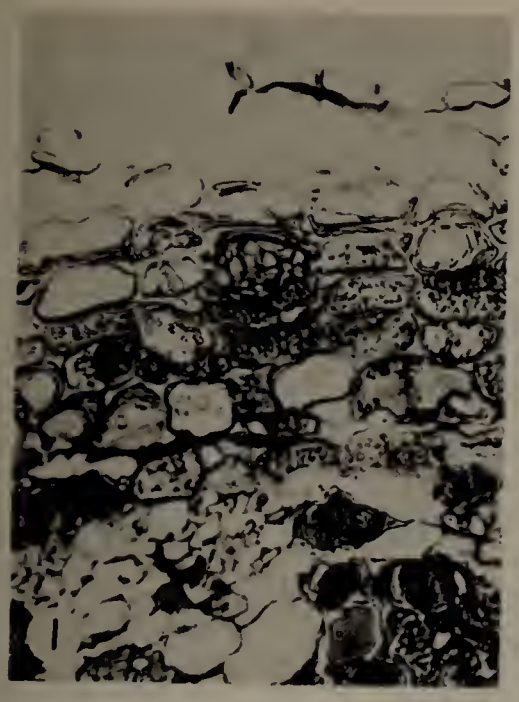

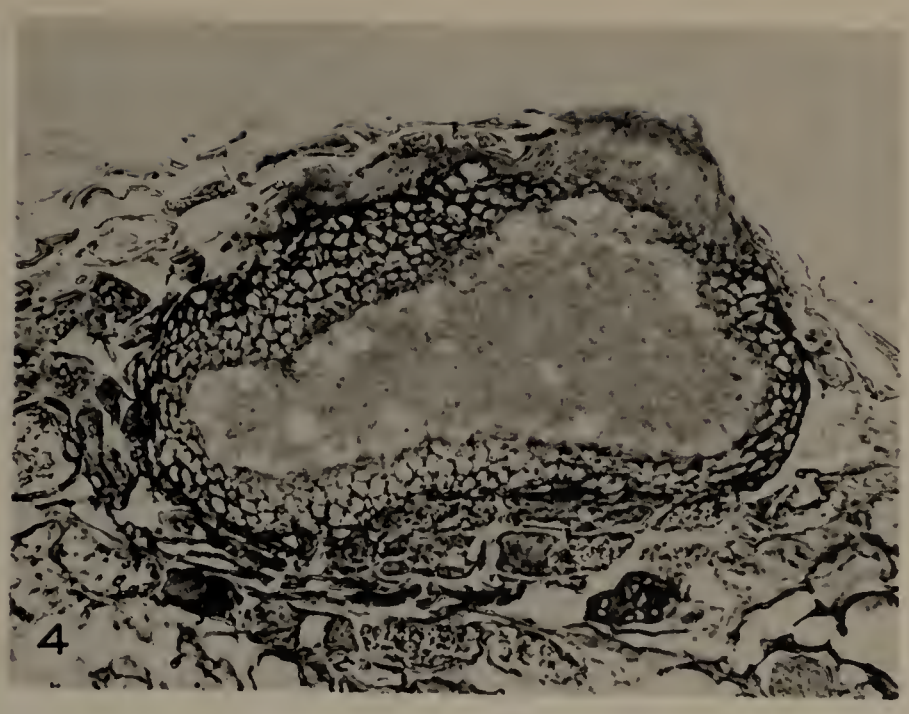

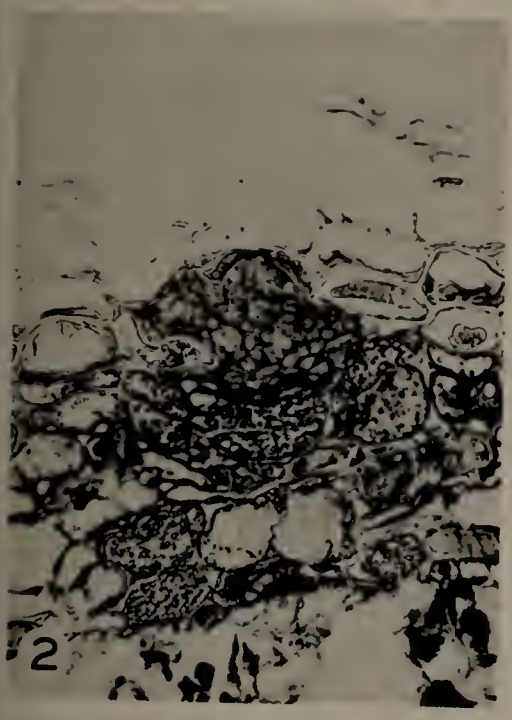

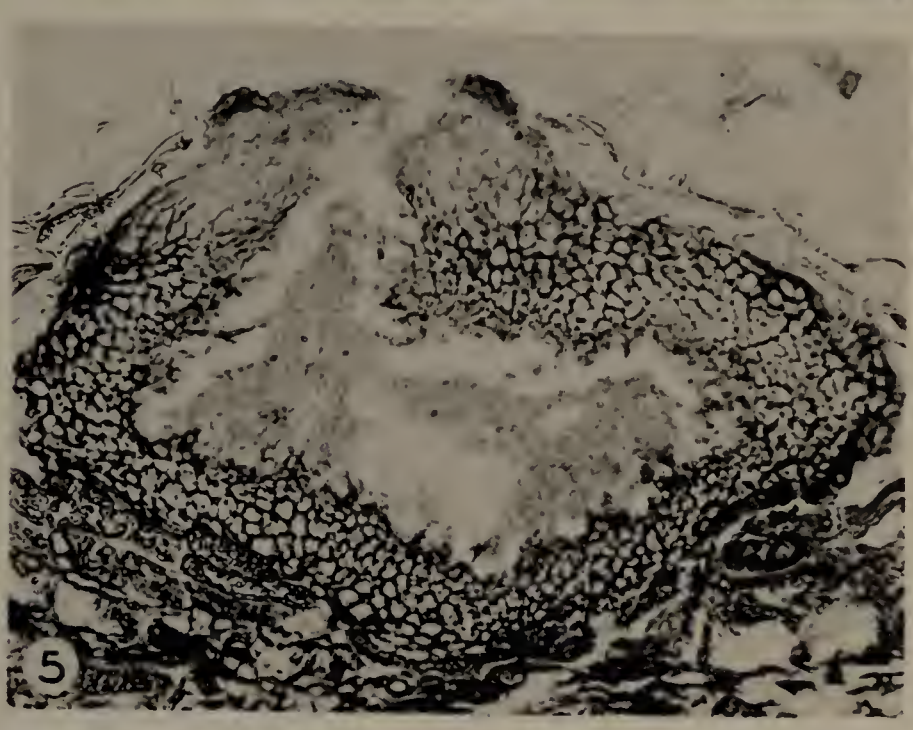

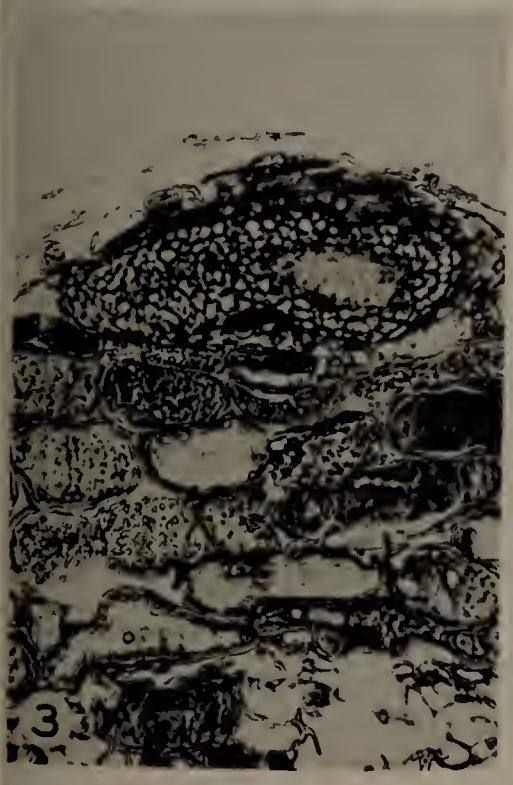

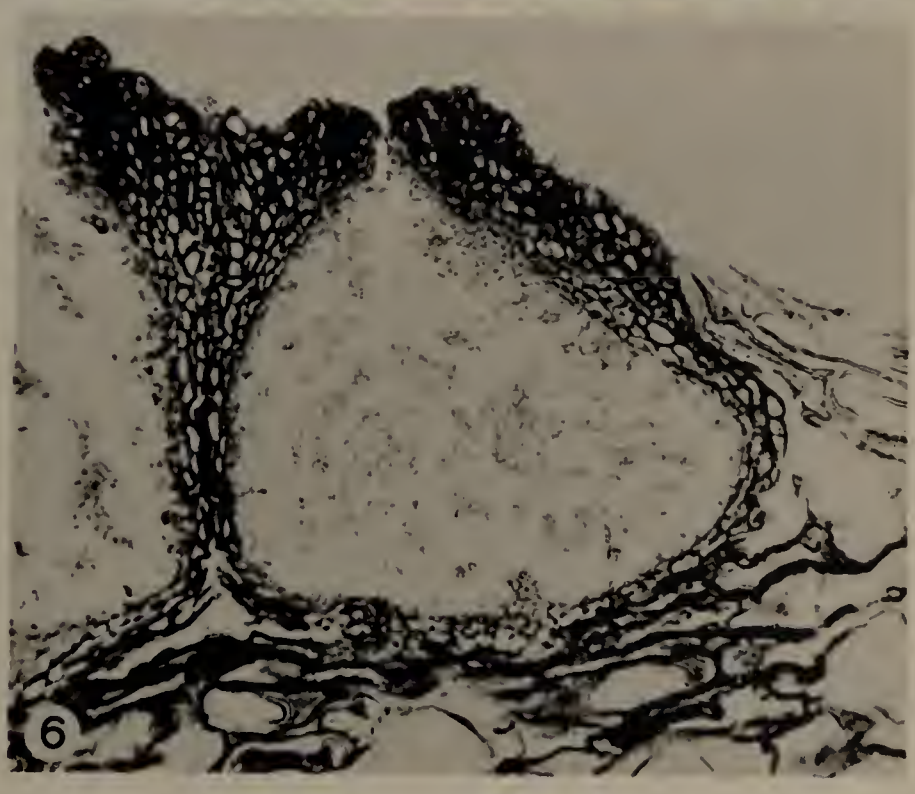

CEPHALOSPORIUM DISEASE OF ELMS

FULL-TONE — MERIDEN

CEPHALOSPORIUM DISEASE OF ELMS

FULL-TONE — MERIDEN

CEPHALOSPORIUM DISEASE OF ELMS

FULL-TONE — MERIDEN

1

2

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CEPHALOSPORIUM DISEASE OF ELMS

FULL-TONE — MERIDEN

1

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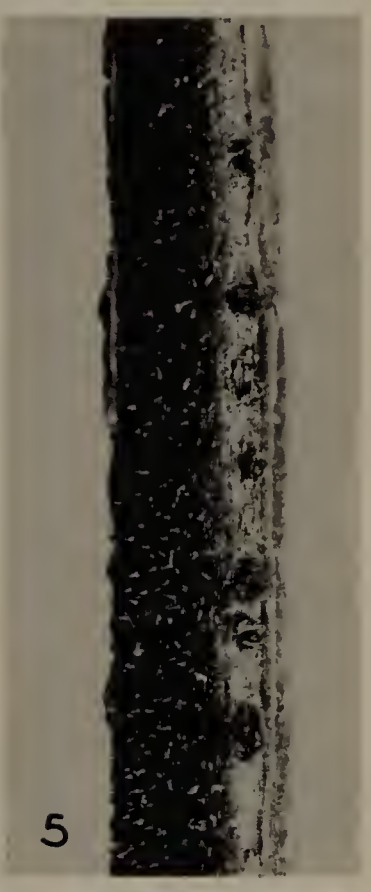
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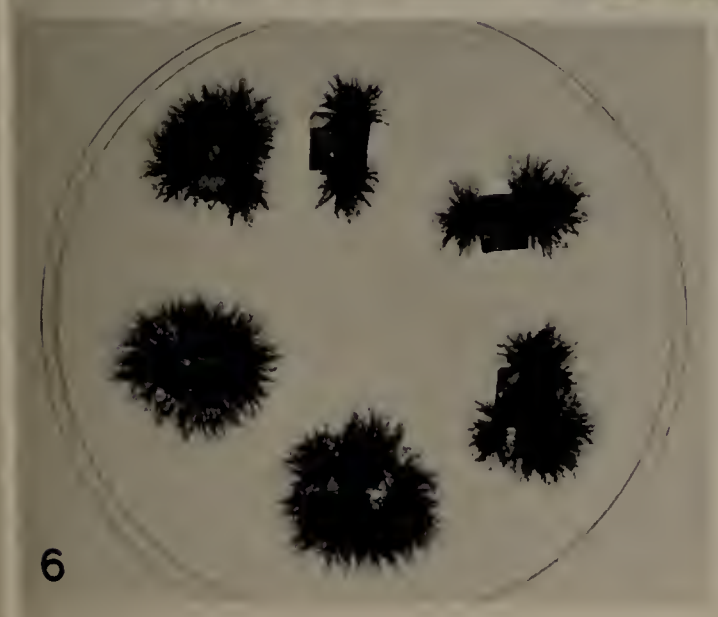
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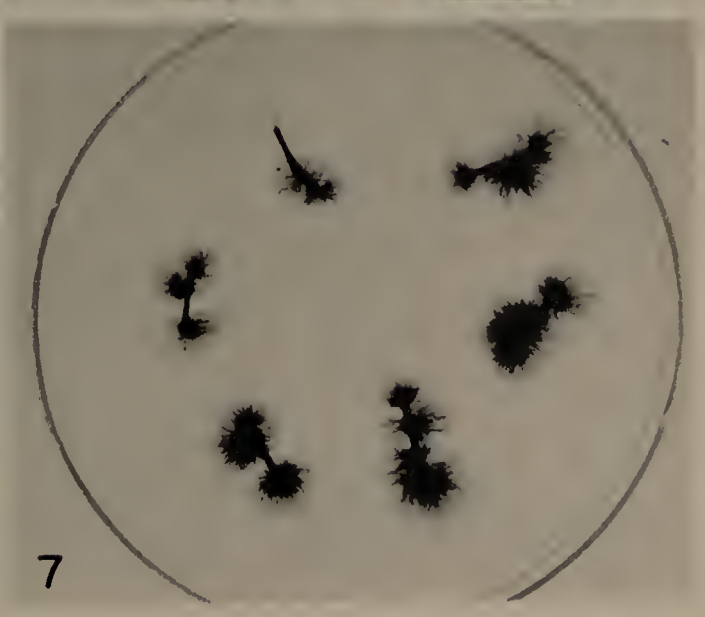
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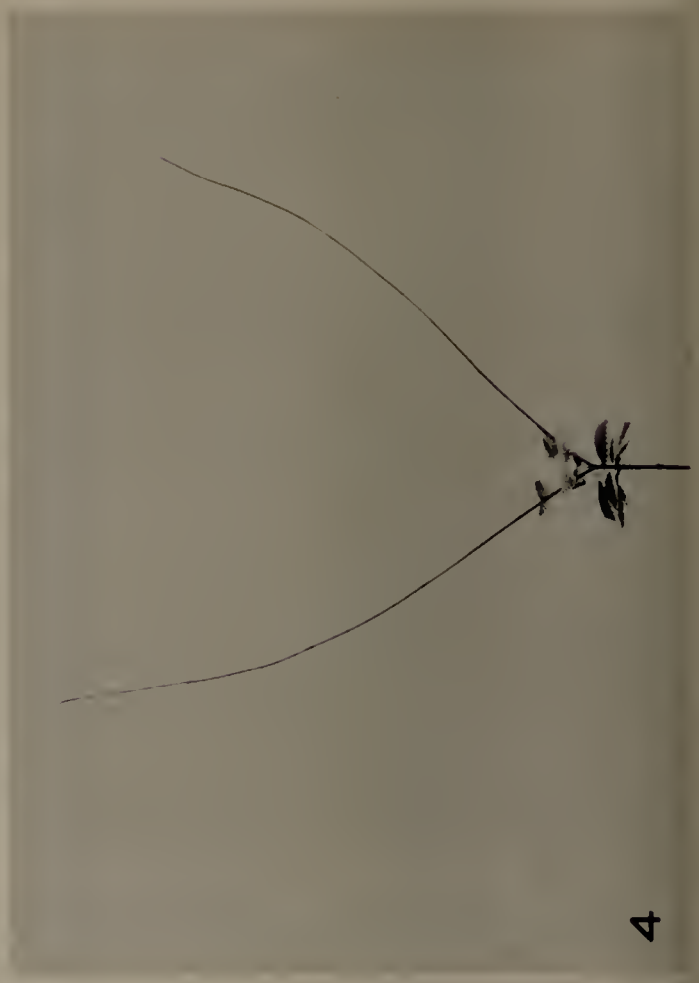
CEPHALOSPORIUM DISEASE OF ELMS

FULL-TONE — MERIDEN



CEPHALOSPORIUM DISEASE OF ELMS

FULL-TONE — MERIDEN

CEPHALOSPORIUM DISEASE OF ELMS

FULL-TONE — MERIDEN

CEPHALOSPORIUM DISEASE OF ELMS

FULL-TONE — MERIDEN

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The Silva of North America; A Description of the Trees which grow naturally in North America exclusive of Mexico. By Charles Sprague Sargent. With 740 plates drawn from nature by Charles Edward Faxon. 14 vols. f°. Boston and New York, 1891–1902. *Price* $350.00

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A Manual of the Trees of North America exclusive of Mexico. By Charles Sprague Sargent. With 783 illustrations from drawings by Charles Edward Faxon and Mary W. Gill. Second edition reprinted with corrections. 910 pp. 8°. Boston and New York, 1926. *Price* $5.00

Manual of Cultivated Trees and Shrubs Hardy in North America exclusive of Subtropical and Warmer Temperate Regions. By Alfred Rehder. 37 + 930 pp. 8°. New York, 1927. *Price* $5.00

Corrections and Emendations of Rehder's Manual of Cultivated Trees and Shrubs. By Alfred Rehder. 19 pp. 1935. *Price* $0.10

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